UNIVERSITY OF CAPE COAST

VIRULENCE GENES DISTRIBUTION AND MOLECULAR CHARACTERISATION OF ANTIBIOTIC RESISTANCE IN *HELICOBACTER PYLORI* INFECTED PATIENTS PRESENTING WITH GASTRODUODENAL PATHOLOGIES IN THE CENTRAL REGION OF GHANA

ERIC GYAMERAH OFORI

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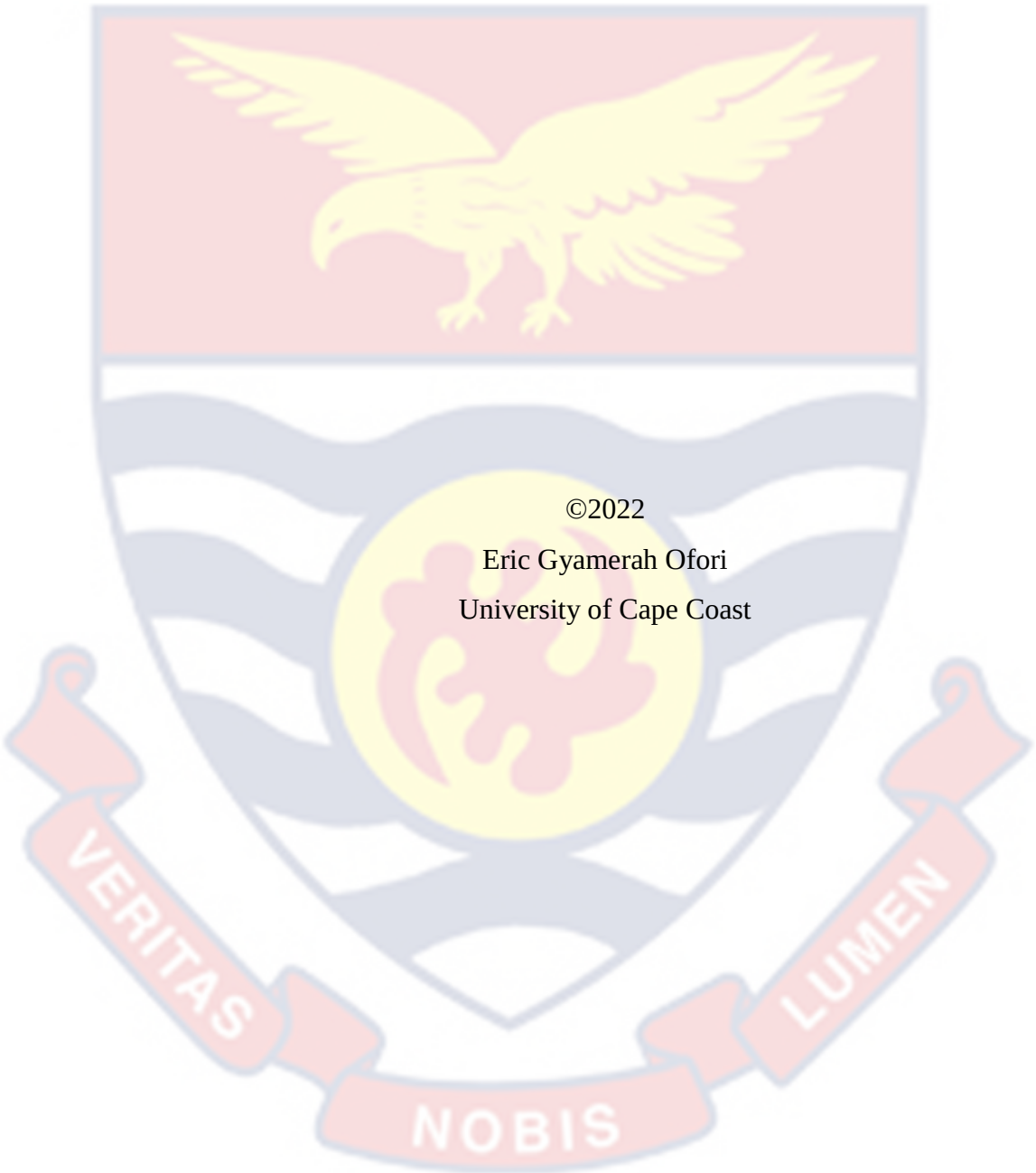

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BY

ERIC GYAMERAH OFORI

Thesis submitted to the Department of Molecular Biology and Biotechnology of the School of Biological Sciences, College of Agriculture and Natural Sciences, University of Cape Coast, in partial fulfilment of the requirements for the award of Doctor of Philosophy degree in Molecular Biology and Biotechnology

DECEMBER, 2022

DECLARATION

Candidate's Declaration

I hereby declare that this thesis is the result of my own original research and that no part of it has been presented for another degree in this university or elsewhere.

Candidate's Signature: ..... Date: .....

Name: Eric Gyamerah Ofori

Supervisors' Declaration

We hereby declare that the preparation and presentation of the thesis were supervised in accordance with the guidelines on supervision of thesis laid down by the University of Cape Coast.

Principal Supervisor's Signature: ..... Date: .....

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Co-Supervisor's Signature: ..... Date: .....

Name: Dr. Foster Kyei

ABSTRACT

H. pylori is considered one of the most successful pathogens known to cause infections of the gastric mucosa. The associated disease outcome and treatment approach is influenced by a number of factors. The current study examined the prevalence dynamics, risk factors, antibiotic resistance and bacterial virulence factors in persons infected with *H. pylori*. Sequencing of genes responsible for antibiotic resistance, PCR amplification of virulence factors and resistant genes were also performed. Information from administered questionnaire were used to determine risk factors. Secondary data from endoscopy referral Centres were analysed for dynamics of prevalence. Infection prevalence was found to be 77.09 % in males and 74.54 % in females. All oesophageal cancer cases were males and gastric cancer cases occurred at 2.8 % in males compared to 0.39 % in females. Stomach and duodenal ulcers, gastritis, hiatal hernias, and esophagitis were all significantly linked to *H. pylori* infection. *iceA2*, *dupA*, and *cagE* were identified at a rate of 28.16 %, 32.04 %, and 12.62 % respectively. The relationship between *iceA2* and conditions such as duodenal and gastric ulcers were statistically significant. Phenotypic resistance rate in levofloxacin, tetracycline, clarithromycin, metronidazole and ciprofloxacin were found to be 40 %, 20 %, 100 %, 100 % and 20 % respectively. A number of mutations in *gyrA* (eg. H200Y, A199V/T, R190S, H189Y and A97V), *rdxA* (eg. Q11Stop, R16H, E27G, I36T. Q50Stop, D61G and A67G), and pbp1A (eg. T254I, K315E and T438M) were identified. Again, amino acid substitutions may lead to structural modification of antibiotic resistance genes. Studies on other virulence factors and resistance gene are required to fully understand the infection.

KEYWORDS

Gastroduodenal pathologies

Clinical condition

Biopsy

Gene

Endoscopy

Amplification

Epidemiology

Therapeutic

Virulence factor

Amino acid

Resistance

Genetic variability

Colonization

Phenotypic

Mutation

Sequence

Synonymous

Non-synonymous

Tertiary structure

Genome

Duodenal ulcer

Esophagitis

Gastric ulcer

Secondary structure

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I want to thank my wife, Rosina Osei Bonsu, and my two wonderful kids, Nana Aba Ofori Achiano and Godwin Ofori Amanfo, for their love. I ask for God's protection over my mother and my siblings. Your prayers have allowed me to get this far. I'd like to send my late father's soul everlasting peace.

DEDICATION

This thesis is dedicated to my family

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LIST OF ABBREVIATIONS

AMC-CA	Amoxicillin-Clavulanic Acid
AMR	Antimicrobial Resistance
AMX	Amoxicillin
BabA	Blood Group Antigen A
BHI	Brain Heart Infusion
CagE	Cytotoxin Associated Gene E
CagPAI	Cytotoxin Associated Gene Pathogenecity Island
CIP	Ciprofloxacin
CLR	Clarithromycin
CM-LAMP	Colorimetric Magneto Loop-Mediated Isothermal Amplification
CRPIA	Conserved Repeat Responsible For Phosphorylation Independent Activity
DU	Duodenal Ulcer
DupA	Duodenal Ulcer Promoting Gene
EIA	Enzyme Immunoassay
EMT	Epithelial-Mesenchymal Transition
FlaA	Flagellin Protein A
GC	Gastric Cancer
GERD	Gastro-Esophageal Reflux Disease
Git1	G-Protein Coupled Receptor Kinase Interactor 1
GNDP	Ghana National Drug Program

GU	Gastric Ulcer
gyrA	Gyrase A
H. pylori	*Helicobacter pylori*
HH	Hiatal Hernia
HpaA	*H. pylori* Antigen A
HSP60	Heat Shock Protein 60
IceA	Induced By Contact With Epithelium
LCI	Linked Colour Imaging
LEV	Levofloxacin
MALT	Mucosa Associated Lymphoid Tissue
MDR	Multidrug Resistance
MLST	Multi-Locus Sequence Typing
MTZ	Metronidazole
NAP	Neutrophil Activating Protein
NBI	Narrow Band Imaging
nlaIIIR	Neisseria Lactamica Type III Restriction Enzyme
NSAID	Non-Steroidal Ant-Inflammatory Drug
pbp	Penicillin Binding Protein
PMN	Polymorphonuclear Leucocytes
PPI	Proton Pump Inhibitor
PUDs	Peptic Ulcer Diseases
RPTP	Tyrosine Phosphate Receptor Protein
RUT	Rapid Urease Test
SAT	Stool Antigen Test

T4SS	Type IV Secretion System
Th	T helper cell
Treg	T regulatory cell
TTC	Triphenyltetrazolium Chloride
UBT	Urea Breadth Test
UreB	Urease Subunit B
VacA	Vacoulating Cytotoxin A
WHO	World Health Organisation
WLE	White Light Endoscopy

CHAPTER ONE

INTRODUCTION

Background to the Study

The bacterium *Helicobacter pylori* (*H. pylori*) is a common microaerophilic organism with 5-7 flagella at one of its spiral shaped end (Versalovic, 2011). It is one of the most successful pathogenic bacterium known to obstinately live in the human gastric mucous layer (Prashar et al., 2022). It is a catalase positive, curved, and motile as well. Known since 1893, it gained recognition in 1982 through a study by Warren and Marshall. (Marshall & Warren, 1984). After its discovery, studies on this organism have gained much attention and interest due to the relationship it shares with several gastroduodenal pathologies. According to Kuna et al. (2019) and Malfertheiner et al. (2017), *H. pylori* is a chronic infectious agent that affects more than 50% of the world's population and causes varied degrees of gastrointestinal illness. The World Health Organization (WHO) has designated it as a class I bacterial carcinogen (Ansari & Yamaoka, 2019). Numerous studies have established that *H. pylori* plays a significant role in the aetiology of peptic ulcer disorders (PUDs), including chronic gastritis, duodenal ulcer, gastric cancer, gastric ulcer, and mucosa associated lymphoid tissue (MALT) lymphoma, among others (Sjomina et al., 2018).

Globally, infection with *H. pylori* is stated to represent about 75 % of non-cardia gastric malignancy (Dixon, 2001). All human populations are at a risk of this infection but persons in the lower socioeconomic class and living in overcrowded and poor sanitary conditions are at a greater risk (Cover et al.,

1997). In view of this, infection is almost ubiquitous to what pertains in unindustrialized countries with a prevalence of about 90% compared to 25% in advanced countries (Hooi et al., 2017; Hunt et al., 2011; Zamani et al., 2018). The majority of infections are acquired in infancy and can last a lifetime. They transmit vertically within a family or tight circle of close companions by oral-oral or faeco-oral contact (Atherton, 2006; Blaser & Berg, 2001). Many infected individuals show no sign and symptom of infection and are found to be positive only after analysis of biopsies. However, close to 20 % of persons infected with this bacterium develop severe pathologies including gastric ulcers and lymphomas (Montecucco & Rappuoli, 2001). Clinical conditions caused by *H. pylori* is influenced by a number of factors including host genetic susceptibility, bacterial strain heterogeneity (with accompanying virulence factors) and host living environment (Amieva & El–Omar, 2008; Forman & Burley, 2006; Šterbenc et al., 2019).

Once it enters the human gastric mucosa, *H. pylori* must survive harsh gastric conditions by evading hydrochloric acid and digestive enzymes, and withstand host humoral and cellular immune responses (Suerbaum & Michetti, 2002). This bacterium is however, difficult to eliminate due to its complex virulence factors and immune evasive techniques, causing lifelong persistence in infected individuals (Atherton, 2006; Sheikh et al., 2018). Strain variability correlates with specific virulence factors which are then translated into the types of clinical conditions that may arise. Extensive research into the relevance of *vacA*, *iceA*, and *cagA* virulence genes in clinical outcomes have been reported (Xue et al.,

2021). Peptic ulcer disease and gastritis are linked to *cagA* while certain subtypes of *vacA* have been linked to prolonged inflammation of the mucosa, advancement of PUD and development of gastric cancer (GC) (Memon et al., 2014; Sheikh et al., 2018). All strains of *H. pylori* carry the *vacA* gene, but there are regional genotype variations (Van Doorn et al., 1999; Xue et al., 2021; Yamaoka et al., 1999). These elements play a role in the differences in the clinical effects of infection in these distinct geographical locations.

Other less exploited virulence factors include *cagE*, *iceA1/iceA2*, *dupA*, *OipA*, *BabA*, etc. There are inconsistent results on the role of *dupA* in disease outcome. However, in most cases, the presence of this gene is found to share a significant relationship with duodenal ulcer (Lu et al., 2005). The allelic variants of *iceA* are also geographically distributed. While Colombia, America, and Europe have mostly *iceA*2 genotypes, places like Japan and South Korea have mostly *iceA*1 types. Both types have been associated with gastric ulcer and gastritis, either chronic active or moderate chronic gastritis (Akeel et al., 2019). Induction of IL-8 and *cagA* translocation are both impacted by *cagE* (Shariq et al., 2015). According to Sougleri et al. (2016), a decline in stem cells and pointers of the epithelial-mesenchymal transition (EMT) is directed by the ineffective translocation of the *cagA* gene. According to Lima et al. (2011), *cagE* detection was correlated with gastric cancer (GC) in India and Thailand at 100% and 93.8%, respectively.

In spite of these difficulties, *H. pylori* infection treatment follows a consistent therapeutic course across geographic boundaries. It involves a three-drug

regimen that consists of a proton pump inhibitor, metronidazole, and either amoxicillin or clarithromycin as the third antibiotic (Megraud & Lamouliatte, 2003; Nishizawa et al., 2009; Sherif et al., 2004). Quadruple therapy is applied when triple therapy fails. Treatment failure is on the increase in all parts of the world due to antibiotic resistance resulting from point mutation in antibiotic genes. The prevalence of antibiotic resistance also varies geographically. This has been linked to the extensive, unrestricted, and indiscriminate use of antibiotics by the general populace (Diab et al., 2018; Vakil & Megraud, 2007). Independent concluding endoscopic studies have established significant gastric pathologies in the Sub-Saharan African region including Ghana (Agha & Graham, 2005; Archampong et al., 2017). Again, recent studies indicate an alarming increase in antimicrobial resistance (AMR) which is affecting pharmacotherapy. To classify transmission across larger human populations, it is crucial to identify *H. pylori* phenotypically and genetically using culture methods. This study aims to provide information on *H. pylori* AMR in Ghana and offer molecular explanations for the unknown AMR in the Ghanaian population. The data will aid policy formulation and interventions to combat the AMR menace in the country.

Statement of the Problem

A major health burden with possible considerable morbidity is imposed on health care systems by *H. pylori* infection. Present therapeutic measures for *H. pylori* related gastric diseases include “triple therapy”, “bismuth quadruple therapy”, “concomitant therapy”, “sequential therapy”, “hybrid therapy”,

"levofloxacin triple therapy", "fluoroquinolone sequential therapy", among others (Chey et al., 2018). The changing approach to treatment has been as a result of challenges faced with complete eradication of infection, recurrence and relapse. Eradication procedures have been by the use of antibiotic and proton pump inhibitors. A number of studies have reported cases of treatment failure involving the use of metronidazole and clarithromycin as part of triple therapy regimen in managing PUDs (Caliskan et al., 2015; Noh et al., 2016; Zeng et al., 2015). Human activities such as poor diagnosis, indiscriminate and excessive use of antimicrobials like metronidazole, and substandard prescriptions are reported to be involved in these failures (Graham & Fischbach, 2010).

Measures to eradicate the infection may be hampered by the scarcity of knowledge regarding the antibiogram and genes responsible for antibiotic resistance by *H. pylori* in Ghana. Again, some virulence factors are yet to be investigated even though they have shown in other parts of the world to be related to specific clinical manifestations. It is also expected that analysis of possible point mutations and their resultant amino acid substitutions with respect to antibiotic target genes in *H. pylori* would assist in discovering novel approaches to treatment. Once treatment approaches have specific targets, there is the added advantage of avoiding the disruption of commensal flora and effectively achieving pathogenic bacteria eradication.

Purpose of the Study

The study considered generating data on the state of antibiotic resistance regarding *H. pylori* infection and assessed the distribution of virulence genes in

gastroduodenal patients visiting reference laboratories for various gastroscopies in the Central region of Ghana.

Research Questions

The current study was guided by the following questions:

1. What is the prevalence of *H. pylori* infection among male and female patients of different age groups who appear with various gastric-related clinical problems, and how has the trend been over the past 7 years?
2. How are gastroduodenal diseases and *H. pylori* infection related?
3. What are the risk factors related to the prevalence of *H. pylori* infection in Ghana's Central Region?
4. What types of *H. pylori* virulence genes predominate in individuals presenting with various *H. pylori*-related gastric illnesses in the Central Region?
5. Can a type of virulence gene in *H. pylori* provide a clue to the possible clinical outcomes associated with patients presenting with gastroduodenal pathologies?
6. Do *H. pylori* strains isolated from stomach biopsies of PUD patients exhibit sensitivity to first-line treatments or resistance to them?
7. Do the genes causing antibiotic resistance have point mutations, and if so, do the changes alter the secondary and/or tertiary structures of the genes?

Objectives of the Study

The research objectives were to;

1. assess the dynamics of the prevalence of *H. pylori* infection in the Central Region of Ghana.
2. investigate the relationship between *H. pylori* infection and gastroduodenal pathologies
3. identify the risk factors linked to *H. pylori* infection rate in Ghana.
4. determine the major virulence genes associated with the type of *H. pylori* found in the region.
5. analyse the association between virulence genes and clinical outcomes of *H. pylori* infection.
6. evaluate the antibiogram of isolates of *H. pylori* and determine the genotypic basis of the antibiotic resistance.
7. identify potential mutations in the AMR-causing genes by sequencing and ascertain possible secondary and tertiary structural modification(s).

Significance of the Study

This study is anticipated to yield a wealth of data on the prevalence of *H. pylori* infection and antimicrobial drug resistance among Ghanaian patients with PUDs. It is also going to generate data on virulence genes and their relationship with clinical outcomes. The study also looks at possible secondary and tertiary structural changes to antibiotic target proteins when the wild types and mutant genes are compared. The information will also serve as a source for needed data for the control and management of *H. pylori* infection.

Limitations of the Study

There are certain limits to the empirical findings reported here, which must be considered. First, two endoscopy centres in the Central Region provided all study samples. This was mostly due difficulties in gaining access to other institutions (ethical issues and consent), the limited numbers of cases handled by some of these facilities, and the need to travel great distances for such small numbers. Secondly, the sensitivity of test kit for *H. pylori* detection could not be taken into account when data from past patient records (secondary data) was being considered.

Again, sampling was done on purpose, and only people who attended endoscopy centres were chosen for recruitment. As a result, these people have a significant likelihood of having an *H. pylori* infection given that they typically visit referral clinics due to a gastroduodenal illness symptom or sign that has already been identified. This suggests that the only way to define prevalence is in relation to people who report having symptoms of gastroduodenal diseases. This circumstance makes it difficult to generalise to the entire population. Furthermore, the number of biopsies sampled for the study was rather small. Even though all patients who visited the facilities for endoscopy were the intended participants, fewer persons were realised over the course of the study's duration, and some of those who were found did not give their consent. Following the application of the selection criteria, several people were also excluded.

The quantity of successfully isolated bacteria samples utilised in the study's sequencing and analysis, as well as isolates for testing bacteria's susceptibility to antibiotics, is another limitation. This also impacts generalisation of findings.

Definition of Key Terms

To avoid misinterpretation, the following explanations of some specific terms used in this thesis are provided:

Endoscopy: The Oxford dictionary defines endoscopy as a procedure in which an instrument is introduced into the body to give a view of its internal parts.

Infection: the growth of pathogens, their invasion of host tissues, and how the host tissues react to the infectious agent and the toxic substances they produce.

Inflammation: The body's natural defense against injury or infection which is characterised by changes in blood flow, increased blood vessel permeability, and movement of fluids, proteins, and leukocytes from blood vessels.

Gastroduodenal pathologies: a wide range of medical conditions, such as cancer and ulcers, that affect the stomach, duodenum, or esophagus.

Antimicrobial resistance: when a microorganism, such as a fungus or bacteria, acquires the capacity to withstand exposure to antibiotics intended to kill them or halt their growth.

Protein structure prediction: the process of inferring a protein's three-dimensional structure based on its amino acid sequence. That is, predicting its secondary and tertiary structure from the fundamental structure.

Gene sequencing: according to the Collins' Dictionary, gene sequencing involves classifying the order in which the elements making up a particular gene are combined. That is, the process of ascertaining the order in which nucleotides within a DNA molecule are arranged.

Gastric biopsy specimen: a piece of stomach tissue that is removed and examined under a microscope, usually for diagnostic study.

Organisation of the Study

Five chapters make up the thesis. The introduction to the study is covered in the first chapter. It includes the background information for the study, problem statement, objective, and significance. The chapter also examines the thesis' organization as well as its limitations and operational definitions of terminology.

Chapter two comprises the literature review. It looks at human microbiota and the historical background to infections from *H. pylori*. It also describes the epidemiology and transmission of infection as the well as some risk factors that have been identified to play a role in *H. pylori* infection. The chapter also discusses the clinical conditions that are associated with infections from the bacterium together with how colonisation and pathogenesis of disease conditions take place. Also, infection complications, virulence factors and oncogenic signalling factors are all discussed in this chapter. Treatment approach to *H. pylori* infection is described as well in this chapter in addition to

the therapeutic measures that have been identified against this infection. The chapter ends with information existing on antibiotic resistance to *H. pylori*.

Chapter three outlines the research methodology used in the study which include how secondary data was obtained from two endoscopy referral centres in Central Region. It also discusses ethical considerations, exclusion inclusion criteria utilised for sample taking and how the various gastric biopsy samples were processed. The chapter further explains procedures involved in DNA extraction, PCR and electrophoresis as well as antibiotic susceptibility testing steps. The concluding part of chapter three describes how sequencing was done, the processes involved in identifying mutations and the online databases that were utilised to predict protein structures of wild type and mutant proteins.

Chapter four covers the results of the study including findings on prevalence of infection, variation of prevalence in various age groups and the differences in clinical conditions associated with infection. The chapter also outlines the findings on virulence factors identified and their association with disease conditions. It also covers results on antibiotic resistance genes, the identified mutations of the genes investigated and the possible protein structure conformational changes resulting from the mutations after predicted structures of the wild type and mutant proteins were compared.

Chapter five covers the discussion of results obtained from the study as well as the summary of findings, conclusions and recommendations.

CHAPTER TWO

LITERATURE REVIEW

The study examined data on the prevalence of *H. pylori* infection in the Central Region of Ghana and also sought to provide information on the state of antibiotic resistance regarding *H. pylori* infection. It also assesses the distribution of virulence genes in gastroduodenal patients visiting reference laboratories for various gastroscopies in the Central region of Ghana. This chapter examines the literature on the subject of the study. It examines available information on the human microbiota and the prehistory of *H. pylori* infections. Additionally, it discusses some of the risk factors that have been found to contribute to *H. pylori* infection as well as the epidemiology and transmission of infection. Along with the colonization and pathophysiology of disease conditions, the chapter also covers the clinical conditions linked to infections from the bacterium. This chapter also covers infection consequences, virulence factors, and oncogenic signaling factors. In addition to the therapeutic methods that have been developed against this infection, the treatment approach for *H. pylori* infection is expounded in this chapter. Information about *H. pylori* antibiotic resistance is provided in the chapter's conclusion.

Human Microbiota and Historical Background of *H. pylori* Infection

There are many different microorganisms, including bacteria, living inside the human body. Over the course of their existence, these bacteria have undergone significant evolution. Their presence is essential for maintaining the health of each host and for the emergence of various diseases. The average number of

cells in a healthy adult person is outnumbered by the human gut microbiota by about a factor of ten (Yang et al., 2009). The gut is estimated to be home to about 500 to 1000 species of bacteria who live there as their normal habitat (Sekirov et al., 2010). Gut macrophages, B and T cells, keep a check on the growth of these microbes to prevent their overgrowth which can lead to diseases (Musso et al., 2010; Sekirov et al., 2010). They do so by exerting their activities on the growing resident microbial numbers as well as invading pathogenic ones. The host immune response to invading microbes usually decreases the viability of the resident microbes creating a wider room for the pathogenic ones that may be present. When this happens, the pathogenic organism is now able to establish itself as a regular organism of the gut. This is particularly so when eradication approaches do not efficiently deal with them (Sekirov & Finlay, 2009; Stecher & Hardt, 2008). *H. pylori* is one elusive proteobacteria that is capable of utilizing this host response mechanism to effectively colonise the human gut, establish itself successfully and initiate disease progression (Sekirov et al., 2010).

In 1982, scientists discovered the bacterium *H. pylori* to be closely associated with peptic ulcer conditions (Marshall & Warren, 1984). This exposé revealed the complexity of gastric microbiome and pathophysiology of the human gastric mucosa. Early experiments by Barry Marshall and Robin Warren showed that the concentration of these microaerophilic, gram-negative bacteria increased with increasing degree of inflammation. *H. pylori* was previously known as *Campylobacter pyloridis.* This is because *H. pylori* was observed to share many

characteristics with Campylobacter. Campylobacter was a known intestinal disease-causing organism whose culture conditions were comparable to the microaerophilic atmosphere required for the culture of *H. pylori* (Kidd & Modlin, 1998). *Helicobacter* became the new name after it was discovered to belong to the gram negative group of bacteria. Again, morphological features of the flagella and a phylogenetic analysis made it clear that it didn't belong to *Campylobacter* species (Romaniuk et al., 1987). The bacterium was consequently appropriately classified as belonging to its own genus, *Helicobacter* (Romaniuk et al., 1987).

Until the relationship with gastric pathologies were detected, it was believed that the release of acid into the stomach was a prime cause of ulcers which meant therapeutic targets were aimed at neutralising the acid released in the stomach. *H. pylori* discovery and the relationship with these gastric pathologies gave a new direction to measures of control of these diseases. Since then, thousands of research on the pathophysiology and clinical problems brought on by *H. pylori* infection have been published. These publications have been created since its re-discovery, and many more are anticipated to be regularly added (Sonnenberg, 2022). *H. pylori* is an S-shaped Gram negative, short helical microorganism that is between 0.5 and 1.0 µm long. It is established primarily in the *pyloric* region of the stomach and causes varying degrees of gastric pathology. A branch of the World Health Organisation known as The International Agency for Research on Cancer, reported that *H. pylori* is a human carcinogen. Based on

epidemiological data, this has been confirmed in more recent studies (Ansari et al., 2018; Murata - Kamiya & Hatakeyama, 2022).

Epidemiology and Transmission of *H. pylori* Infection

H. pylori has been established in several hosts and from varying environmental sources including blood, faeces, dental plaque, and water among others (Cellini et al., 1995; Falsafi et al., 2007; Huang et al., 2006). The culture from water, dental plaques and other environmental sources however, have been quite challenging leaving molecular methods as the procedure of choice for detection (Isaeva & Isaeva, 2020). Without a precisely known route of transmission, the common source of infection have been thought to be by faecal–oral or oral–oral via contaminated food or contaminated water (Percival & Thomas, 2009). Epidemiological evidence also suggests that the infection is largely acquired during childhood where social amenity deprivation and crowded living conditions appear as significant risk factors (Smith et al., 2019). Pathologies eventually become more noticeable after years of chronic infection and may vary from one geographical location to another (Covacci et al., 1999; Rowland & Drumm, 1998). Across various age groups, reports indicate a rate of 73.2% for ages between 14 and 30 and 71.5 % for ages between 31 and 45 years. The remaining are 68.6 % for the age range of 46 to 60 while 70.40 % has been obtained for those from 61 years to 88 years (Uyanıkoğlu et al., 2012).

Transmission among adults is not so common and this is something that cannot be readily explained. Tourists and missionaries as well as adults living in endemic countries do not become infected and same can be said of health

workers who treat patients infected with *H. pylori* (Go et al., 2005; Lin et al., 1998; Matsuda & Morizane, 2005; Peters et al., 2011). Such health workers are not classified as persons with extremely high occupational risk for *H. pylori* infection acquisition (Lin et al., 1998). The assumption is that, the transfer of infection occurs from parent to child or between children which is thought to occur via the oral-faecal route. It must be noted however, that the rate of transmission is found to be higher among persons of low socioeconomic status. Public health standards in specific habitats are therefore important in trying to explain the widespread of infection transmission between different populations and between individuals of different socioeconomic groups (Genta et al., 2017; Moayyedi et al., 2002). The exact mechanisms and behavioural pathways leading to these are however farfetched (Sonnenberg, 2022).

The distribution of *H. pylori* varies not just between countries but even within a single country, according to a 2013 report by (2013) Around 4.4 billion people worldwide are estimated to be infected with *H. pylori* based on local prevalence assessments, although additional research is required to confirm this estimate. This implies that about 60.30% of the global population are positive for *H. pylori* infection (Hooi et al., 2017). Out of the infected, 80 % occur in developing countries while 20 % are in developed countries (Sanz-Peláez et al., 2008; Singh & Ghoshal, 2006). High incidence in developing nations and the development of *H. pylori*-associated illness are inconsistently correlated. According to studies conducted in Europe and the US, people with *H. pylori* infection are more likely to develop gastritis, stomach ulcers, and gastric cancer

than they are in African nations (Ferlay et al., 2007; Malaty et al., 2002). This is referred to as "The African Enigma" and is mostly still a mystery. Reviews have revealed that the prevalence of pathologies of the gastric mucosa, including gastric ulcers and cancers is comparable in both Europe and Africa (Kidd et al., 1999). Strangely, isolates from Africa are those that commonly possess the main risk factors pervasive for cancer development, a situation that further adds on to the enigma (Asombang & Kelly, 2012; Harrison et al., 2017). This puzzling situation has been analysed to possibly arise from three main triggers; (1) the propensity of an *H. pylori* strain to cause cancer, (2) the influence of co-infections towards Th-2 type of immune modulation, (3) and the relatively higher antioxidant content in the diets of Africans (Ghoshal et al., 2010). All these are known to play prominent role in the extent to which *H. pylori* infection affects humans. Indigenous variances in infection prevalence occur where there has been considerable immigration from countries with high prevalence. A review by Hooi et al. (2017) concluded the distribution of worldwide *H. pylori* prevalence to be 79.1 %, 63.4 %, 54.7 %, and 37.1 % for Africa, Latin America and the Caribbean, Asia and Oceania respectively.

Risk Factors of *H. pylori* Infection

H. pylori remains a major risk factor for PUD and with the increasing prevalence of infection worldwide, the decline in the development of such clinical illnesses may not be in sight anytime soon (Feldman et al., 2020; Zhang et al., 2014). Various risk factors are connected to *H. pylori* infection. They include a lower socioeconomic status and unsanitary conditions or crowding.

These are general factors that make this infection more of a disease for the poor and for those in unhygienic conditions. It is therefore not surprising to find an over 80 % prevalence in Africa compared to 30 – 50 % in adults of advanced countries (Hunt et al., 2011). Again, variations exist in prevalence within a country and across different ethnic groups as evident in over 60 % rate in Mexican Americans and compared to 30 % in non-Hispanic white population (Grad et al., 2012). To prevent or reduce *H. pylori* infectious diseases, it is important to begin by preventing infection and remaining negative for a long time. Analysis of risk factors is an important area that can give an understanding to the channels of reinfection after a successful eradication. It will also suggest effective eradication therapies as well as manage recurrence after primary eradication.

Further studies aimed at identifying contributing factors to *H. pylori* infection have been presented. A study in Portugal on 166 adults obtained a high infection prevalence in participants with lower vegetable/fruit intake in addition to preference for fried foods. The use of well water, inappropriate hand washing practices were also identified as risk factors in the study while frequent (>5 days/week) honey consumption had a negative correlation with *H. pylori* prevalence and reduced *cagA* seroprevalence (Venneman et al., 2018). Although *H. pylori* has been identified in stool samples, the possibility that houseflies can spread these bacteria was not explored until an intriguing theory in this regard was published by Junqueira et al. (2017) in Brasil. The authors suggested a possible *H. pylori* transmission by blowflies and houseflies as they

get into contact with infected faecal matter. Even though the bacterium was identified in about 116 individual blowflies and houseflies from Brasil, there was no further examination to ascertain their viability. In Iran, Siavoshi et al. (2018), found *H. pylori* specific *16S* rDNA in yeast cells that were isolated from honey and honey bees, fruits, flowers and oral swabs from villagers. The study detected *H. pylori* in 65 % of the yeast cells with the majority found in the flower and fruit group (83.3 %), as well as the honey and honey bee group (83.3 %).

Genetic Variability

Various adaptations are adapted by *H. pylori* to cope with the harsh environment of the gastric mucosa. The need to persist means a lot of genetic recombination resulting in various variability in strain. Of the 1186 genes possessed by this organism, 22 of them constitute genes for a particular adaptation to the human gastric environment (Lara-Ramírez et al., 2011). A great deal of research has focused on complete sequencing of the full genome of *H. pylori* due to the relevance of the findings in clinical practices. This identified over 200 complete strain genomes described in various databases (Cao et al., 2016; Thorell et al., 2017). The probability of identifying isolates of variable genetic make-up is very likely if the isolates are from different subjects who are unrelated in several ways (Akopyanz et al., 1992). Among all bacteria, *H. pylori* remains the most studied so far. It has the highest genetic recombination resulting from mechanisms such as point mutations, strain specific restriction modification, chromosomal rearrangements, and impaired DNA repair mechanisms (Aftab et al., 2017; Blaser & Berg, 2001; Falush et al., 2001; Suerbaum & Michetti,

2002). Owing to the easy transformation characteristic possessed by *H. pylori*, it is relatively easier to take up subsequent homologous recombination results in the diverse genetic multiplicity. DNA fragments of foreign origin undergo recombination for integration in bacteria chromosomes especially when they have relatively free foreign DNA. When more than a single strain of this bacterium exists together in an individual's stomach, an exchange of genetic material can occur (Ailloud et al., 2019; Falush et al., 2001). This microorganism is considered a reliable marker for the evolution of prehistoric migration of humans. This is because it is believed to have been in the stomach for millions of years and the diversity of sequences at selected gene sites are related to certain human migrations (Farzi et al., 2019; Hanafiah & Lopes, 2020).

H. pylori can be grouped into eight populations based on geographical distribution as hpEastAsia, hpAsia, hpAfrica1, hpAfrica2, hpNEAfrica, hpEurope1 (AE2), hpEurope2 (EA2), and hpSahul (Breurec et al., 2013; Falush et al., 2003; Matsunari et al., 2012). Organisms with the different geographical origins of strains are associated with differences in chronic atrophic gastritis, gastric adenocarcinoma, and duodenal ulcers. For example, since the strains in East Asia (areas around Korea and Singapore) are distinct from those of Europe, clinical conditions in these areas are expected to accordingly correspond to this variation. Thus, genetic studies into *H. pylori* infection is an essential investigation for clinical investigations. The fact that the incidence of stomach cancer is lower in Asia and Africa, where infection rates are higher, provides a

strong indication of the problem. This may be resulting from the presence of a particular strain unique to these geographical regions.

H. pylori genotyping includes random polymorphic DNA amplification, multi - locus sequence typing (MLST), pulsed - field gel electrophoresis, whole - genome sequencing and amplified fragment length polymorphism. MLST is based on allelic mismatch using polymorphisms in seven housekeeping genes (Vazirzadeh et al., 2022). Though expensive, this method is rapid, reliable and provides high resolution and repeatability. It also gives an additional advantage of providing comprehensive information about human genetic analysis which can be mapped to human migration to some extent (Bie et al., 2019; Keikha, 2020; Vazirzadeh et al., 2022). This method together with the Structure Bayesian population cluster studies have been applied in the documentation of the seven distinct *H. pylori* populations (Vale et al., 2015).

H. pylori Infection and Associated Gastroduodenal Pathologies

H. pylori prevalence in the spectrum of gastritis

Nearly 90% of *H. pylori* infections are asymptomatic and acute gastritis is brought on by a new infection. Inflammatory cell infiltration is a result of *H. pylori*'s early activity and colonization of the gastric mucosa. This is the outcome of polymorphonuclear leukocytes' (PMNs') infiltration of the stomach's epithelial layer. The mucosal contact causes dyspeptic symptoms such as nausea, vomiting, and a feeling of fullness. When this infiltration continues for a while, a chronic inflammatory stage is reached. The most frequent histological manifestation of *H. pylori* stomach infection, chronic

active gastritis, develops most frequently from the acute phase. Chronic active gastritis is defined by plasma cells and lymphocytes infiltration while PMNs are involved in the acute phase (Dixon et al., 1996b). Long-term infection can lead to intestinal metaplasia or gastric atrophy in a subset of patients.

H. pylori prevalence in the spectrum of duodenal ulcer

Duodenal ulcer results from severe inflammation by *H. pylori* infection that is confined to the antrum of the stomach. This is probably due to the secretion of too much acid into the duodenal lumen. Following ingestion, *H. pylori* binds to the duodenal and stomach epithelial cells, impeding regular physiological processes. This leads to elevated gastrin release and uncontrolled gastric acid secretion into the lumen (Graham & Go, 1993). This endogenous hypersecretion of gastric acid, bacterial action, and associated inflammatory response predisposes the duodenal mucosa to ulceration (Carrick et al., 1989). *H. pylori* was recognized as the sole causative agent and a strong risk factor (RR=51) (Carrick et al., 1989), (OR=4) for duodenal ulcer pathogenesis in 44.1% of subjects (Cekin et al., 2012; Nomura et al., 1994). According to the aforementioned study, 84.9% of the patients with duodenal ulcers had an infection (Cekin et al., 2012). Another study discovered that *H. pylori* was present in 99.1% of people who had duodenal ulcers (Carrick et al., 1989; Gisbert et al., 1999; Reinbach et al., 1993). *H. pylori*-negative patients also had significantly worse clinical outcomes with eradication therapy over 2 years (Bytzer et al., 2001). When infected, duodenal ulcers may remain healed after a decrease in acid secretion and recur after eradication (Hobsley et al., 2008).

H. pylori prevalence in the spectrum of gastric cancer

H. pylori is identified to cause up to 592000 cases of gastric cancer in Eastern Europe, Eastern Asia, in addition to Central and South America (Parkin, 2006). The increase in gastric stem cells, loss of pepsinogen production activity by zymogen cells and a loss of acid producing parietal cells is a marker for atrophic gastritis (Suerbaum & Michetti, 2002). Prolonged infection means an eventual loss in architecture of the gastric lining and transition towards metaplasia and fibrosis. For *H. pylori* patients with chronic gastritis, there is a 50 % chance that such individuals will develop atrophic gastritis (Kuipers et al., 1995). Atrophic gastritis and stomach cancer are connected physiologically, according to a cancer study. It was discovered that those with *H. pylori* infection had a 10-fold increased chance of acquiring stomach cancer (Kusters et al., 2006). The chance of acquiring stomach cancer is affected by a variety of other factors. Although smoking, host gene polymorphisms, and dietary variables all have a role in chronic atrophic gastritis, the condition is still remains a precursor.

Colonisation and Pathogenesis of *H. pylori* Infection

H. pylori makes use of a number of immune subversion mechanisms and virulence factors in order to live and thrive in the hostile environment of the gastric mucosa. Luminal colonisation in the stomach is severely minimal because of the resultant pH (1-2) from the presence of gastric acid production. This is in contrast to the close-to-neutral environment in the lower parts of the bowel. The stomach is only able to support *H. pylori* for a brief length of time before migrating quickly to the epithelium, where the immunological damage

is less severe (Tille, 2015). The organism is protected from gastrointestinal secretions by the pH change. The availability of flagellar facilitates motility and enables the bacterium to enter and colonise the area of the mucosa close to the epithelium. The viscoelasticity properties of gastric mucins which make the mucosa appear jelly enough to trap bacteria is lowered when the pH is raised to near neutral levels (Celli et al., 2007; Celli et al., 2009). The reduction in pH is made possible by *H. pylori* urease-catalysed action that produces ammonium ions, raising the pH and subsequently transitioning the mucous gel into a solution (Montecucco & Rappuoli, 2001). With the help of the flagellar, *H. pylori* can easily swim within the polymer solution and move to the epithelium where it colonises.

Chemical signaling trigger the movement of *H. pylori* towards a particular end of the epithelium. *H. pylori* has membrane-bound chemoreceptors (TlpA, TlpB, and TlpC) alongside a cytoplasmic chemoreceptor (TlpD) (Lertsethtakarn et al., 2011) through which they sense and incorporate signals for colonisation. Due to the differences in type and concentration of chemoreceptors at different locations of the stomach, *H. pylori* localization and proliferation may differ from one point in the stomach to the other. While chemotaxis in the corpus is for initial localization, the antrum and antrum-corpus zone chemotaxis promotes bacterial proliferation (Valenzuela et al., 2003). Swimming abilities of *H. pylori* are very critical to bacterial survival. Research has shown a reduction in mucosal colonisation capacity in *H. pylori* isolates with observed mutations in genes responsible for specific flagellar proteins such as FlaB, FlaA, and FLA

(Forbes et al., 1995). A close association with the epithelium promotes established colonization while exacerbating more inflammation. Progression of inflammation is directly related to a decrease in microbial populations (Sayi et al., 2009), and this situation implies that the organism must gradually manage its interactions with the gastric epithelium to avoid complete extinction. According to arguments put out by earlier studies, acid activated *VacA* can integrate into eukaryotic lipid bilayers and plasma membranes to create anion and urea-selective membrane channels (Czajkowsky et al., 1999). By giving urease a substrate, the action of urea permease can improve acid survival (Atherton, 2006). Additionally, another investigation found that *VacA* increased epithelial barrier permeability, most likely through acting at the tight junction level (Papini et al., 1998).

Further gastric mucosal colonisation factors have been revealed as contributors of obtaining an optimal niche for *H. pylori* survival. They include non-*CagA* effects of the secretion system, diet, level of acid secretion and effect of extracellular effector gene, *CagL* among others (Kavermann et al., 2003). These are strain and host characteristics that influence intragastric distribution and long term severe inflammation (type B inflammation) of affected areas (Suerbaum, 2000). Chronic inflammation brought on by an organism is thought to promote the growth of B cells, and ultimately lead to B-cell lymphoma (Levinson et al., 2022).

H. pylori Infection and Complication

The colonization of the stomach mucosa by *H. pylori* causes acute inflammation and damage to the gastric epithelium. Following inflammation, a multitude of disease conditions with varying degrees of severity might develop. The severity might range from mild to chronic atrophic gastritis, stomach ulcers, and finally gastric cancer Kavermann et al. (2003). Histological gastritis is a common complication of *H. pylori* infection, however only a tiny proportion of patients develop clinically significant problems such peptic ulcer disease and stomach cancer (Yamaoka et al., 1999). The host's immune system, the virulence of the *H. pylori* strain, and the presence of outside stimuli (stress, food, degree of cleanliness, or co-infection) are only a few of the many factors that determine the form and severity of the disease (Krzyżek & Gościniak, 2018). According to Crabtree et al. (1991), infections have a strong connection to peptic ulcer disease and non-autoimmune chronic gastritis Crabtree et al. (1991). A great deal of *H. pylori* infections are frequently asymptomatic and manifest no symptoms at all. The majority of the symptoms and signs of this condition, however, are caused by stomach ulcers or duodenal inflammation. Additionally, various gastrointestinal conditions can be implicated for symptoms such vomiting, nausea, and abdominal discomfort (Abbas et al., 2018). Besides, extragastric symptoms associated with cardiovascular, cutaneous, neurological, immunological, hematological, hepatobiliary, respiratory, endocrine, and metabolic issues have all been linked to *H. pylori* Zamani et al. (2017).

Virulence Factors and Oncogenic Signalling

Vacuolating cytotoxin A (*vacA*)

Nearly all strains of *H. pylori* produce *vacA*, which is the second most well researched *H. pylori* virulence factor after *cagA*. It is to be noted also that nearly all *H. pylori* strains carry the *vacA* gene (Atherton, 2006). The name stems from the induction of vacuoles in host cells as a result of the uptake of *VacA* toxin into endosomes. Vacuolization is dependent on recognition by receptor proteins, particularly tyrosine phosphatase RPTPα and RPTPβ (Sewald et al., 2008). Increased phosphorylation of pro-inflammatory p38 kinase is brought on by *vacA's* binding to RPTP (Nakayama et al., 2006) and G-protein coupled receptor kinase interactor 1 (Git1). This up-regulation results in epithelial cell detachment followed by ulceration (Hammer et al., 2015). While the actual role of RPTPα surface receptors remains unclear, studies have shown a reduction in vacuolating activity of *VacA* in the absence of RPTPα (Yahiro et al., 2003).

Apart from vacuolization and direct oncogenic signalling, *vacA* is involved in other activities relating to *H. pylori* infection. It causes membrane channel formation, affects mitochondrial function, and induce pro-inflammatory signalling. The ability to traverse the membrane allows it to influence mitochondrial activity and cause apoptosis in epithelial cells (Isomoto et al., 2010). When *cagA* and *vacA* are examined closely, they are found to be somehow connected and to have a number of antagonistic interactions. For instance, *cagA* can prevent *vacA* from causing cells to undergo apoptosis and lessen its vacuolising effects (Akada et al., 2010; Argent et al., 2008). *vacA* on

the other hand is known to inhibit cellular elongation induced by *CagA*. *vacA* can also prevent the activation and proliferation of T lymphocytes, affect Blymphocyte antigen performance, and modulate the Tcell-mediated cytokine response (Gerbert et al., 2004). This toxin can form large, water-soluble oligomeric complexes (consisting of 12 or 14 identical monomers) or it can break down into monomers depending on the pH of the medium. Neutral pH promotes congregation while an acidic or basic pH stimulates fragmentation (Cover et al., 1997). Secreted *vacA* toxin is transformed into a pro- transporter protein that undergoes N and C-terminal cleavage. A fully-fledged secreted *vacA* toxin consists of 2 subunits namely p37 and p58. These units consist of close to 821 amino acids that are connected by way of a flexible loop region (Domańska et al., 2010; Lee et al., 2002). The p58 domain affects binding of the toxin to cells while the intracellular activity is mediated by an amino-terminal domain (Papini et al., 1998; Yeh et al., 2010).

Heterogeneity exists among *vacA* alleles and clinical variations occur for different allelic variants. Research into these variants is therefore very important in appreciating the varying manifestations of clinical outcomes among *H. pylori*-infected subjects. Alleles of *vacA* are either type1 or type2. Several reports have shown that individuals infected with the type1 allele of *H. pylori* have an increased risk of developing gastric ulcers (Atherton, 1997). The variable *vacA* subtype can be divided into three parts. The signal area (s region), which is located at the end of the 5' region, the middle region (m region), and the intermediate region (i region) make up these three regions. All three alleles

can be identified as *s1*/*s2*, *m1*/*m2*, and *i1*/*i2* (González-Rivera et al., 2012). Other researchers have identified an i3 type of the intermediate region, different genotypes of the s1 region as s1a, s1b, s1c and an additional sub-genotype for m1 as m1T (Palframan et al., 2012). Type 1 alleles are the most virulence in initiating apoptosis. Initiation of *vacA* apoptosis is stimulated via a reduced expression of connexin 43 (Cx43) which is directly involved in cell-to-cell communication (Radin et al., 2014). Induction of apoptosis takes place when *vacA* toxin is moved to mitochondria (Isomoto et al., 2010). Depending on what *vacA* toxin binds to or where it is deposited, different cellular responses may be associated with it. For instance, when *vacA* binds to cell surfaces, pro-inflammatory reactions are triggered, and when *vacA* crosses tight junctions, T and B cell activation and proliferation are regulated. Again, endocytosis of the bioactive form of *vacA* results in the formation of membrane channels. This endocytosis is also associated with cytoplasmic vacuolation and leakage of nutrient from epithelial cells of the gastric mucosa (Ivie et al., 2010).

Cytotoxin associated gene pathogenicity island *(CagPAI and cagA/cagE genes)*

The *H. pylori cagPAI* is a virulence factor that has about 27 to 31 genes. It was first identified in relation to human cancer and encodes close to 145 kDa. *cagPAI* is not always identified as an intact gene in all isolated strains of *H. pylori*. For instance, 12 % of isolated *H. pylori* strains in Indian population habour a complete *cagPAI* gene, while strains from Peru possess 18.1% of an intact *cagPAI* gene. The prevalence of an intact *cagPAI* in Japan is 57.1% while

more than 70% have been identified in both Malaysia and Singapore (Kauser et al., 2004; Schmidt et al., 2010). Generally, *cagPAI* can be put into two main groups namely *cagI* and *cagII* separated by insertion sequence of IS601 (Lai et al., 2013). Comparison between *H. pylori* strains with deleted sections of genes in *cagPAI* and those with intact genes has shown an association between severe gastric diseases and *cagPAI* with all genes intact. All genes were discovered to be significantly linked to an elevated risk of gastric cancer (GC) (Ahmadzadeh et al., 2015).

Cytotoxin-associated gene A (*cagA*) and cytotoxin-associated gene E (*cagE*) make up the majority of *cagPAI*. The *cagE* of *cagPAI* is associated with *cagA* and impacts *cagA* translocation as well as IL-8 induction (Shariq et al., 2015). The ineffective translocation of *cagA* protein is a sign of a reduction in stem cells and epithelial-mesenchymal transition (EMT) markers (Sougleri et al., 2016). EMT is associated with improved expression of CD44 and increased cell invasion, cell migration and tumour-like sphere formation (Bessede et al., 2014). Both play a significant role in *cagPAI*, although *cagE* has been found to be a more reliable marker for determining the full existence of *cagPAI* (Khatoon et al., 2017). However, nothing is known about the precise function of *cagE* genotypes as a biomarker of gastrointestinal disorders. The interaction and relationship between the *cagE* and *cagA* genes of the *cagPAI*, as well as their coexistence in patients with gastric cancer observed in high risk populations, are therefore the foundation for an adequate assessment (Bakhti et al., 2020).

CagE positive subjects are the only individuals who are capable of promoting EMT by the upregulation of TGFβ1-mediated EMT pathway (Chang et al., 2015). Research conducted in Turkey (81.8%), India (100%) and Thailand 93.8%) have recorded higher incidence rates of *cagE* gene in GC patients (Lima et al., 2011). On the contrary, other studies found no association between *cagE* and clinical outcome (Dabiri et al., 2017; Dadashzadeh et al., 2017; Lima et al., 2011). Geographical areas with varying degrees of GC incidence such as South-Central Asia, South Eastern Asia, Eastern Asia, and South America (with rates 6.7, 6.0, 24.2, and 10.3 respectively) have indicated no association between *cagA* and *cagE* with gastric cancer (Ferlay et al., 2015; Khatoon et al., 2017).

The *cagA* gene is located at the 3' end of *cagPAI* and encodes a *cagA* protein of about 128-kDa (Covacci et al., 1993). Based on a repetitive sequence of amino acids known as EPIYA at the 3' end of *cagPAI*, *cagA* can be grouped into two types namely the Western and East Asia *cagA*. Western and East Asian *cagA* are usually isolated from Western and East Asian countries respectively. They have different polymorphisms and their level of virulence is also different. East Asia type EPIYA motifs have an A-B-D sequence while the Western type has an A-B-C sequence with a repetitive EPIYA-C that repeats more than once (Higashi et al., 2002). The EPIYA protein motifs of *cagA* gene (glutamine proline-isoleucine-tyrosine-alanine) undergo phosphorylation with tyrosine phosphatase by type IV secretion system (T4SS) once they get injected into cells. The ability to inject substances into cells for pathogenesis is made feasible by the a5b1 integrin receptor's attachment to the *cagL* protein on epithelial cells

(Kwok et al., 2007). The ability to inject substances into cells for pathogenesis is made feasible by the a5b1 integrin receptor's attachment to the *cagL* protein on epithelial cells (Kwok et al., 2007). All isolated *H. pylori* strains from countries like England, Ireland, the United States, Italy, Australia, and Austria have Western-type cagA, but those from high-risk gastric cancer populations in nations like China, Japan, and South Korea have East Asian c*agA*.(Mohamed et al., 2009; Suzuki & Mori, 2016). Non phosphorylation mechanism of action of *cagA* pathogenesis has been reported. This involves interaction of a conserved motif in *cagA* called CRPIA (conserved repeat responsible for phosphorylation-independent activity) with Meth and subsequently by interaction with PI3-K/Akt. This activates NF_kB and β-catenin for the IL-8 secretion (Segal et al., 1996).

Duodenal ulcer promoting gene A (*dupA*)

Elements such as bacterial virulence factors, environment, and host genetics are important influencing elements involved in the nature of the clinical manifestations linked to *H. pylori* infection. *vacA* and *cagA* are two major virulence factors that are well known for their roles in disease initiation and progression. In general, these factors have some bias towards specific conditions, but none are closely related with duodenal ulcers in all populations. The continual search for strain specific genes especially those outside the *cagPAI* region is therefore of significant importance. Global epidemiological results have shown the involvement of *dupA* in IL-8 secretion (Lu et al., 2005).

Increase in IL-8 is as a result of increased secretion from the gastric antrum which leads to antral gastric inflammation.

Antral gastritis is a well-known indicator for duodenal ulcer disease (Shiota et al., 2012). *dupA* gene is located in the plasticity zone of *H. pylori* and homologous to *virB4* gene. Due to this homology, *dupA* forms a type IV secretion system with *vir* genes. It was found to contain two open reading frames, namely *jhp0918* and *jhp0917* with twelve bases overlapping. It is also identified by an insertion of cytosine or thymine after position 1385 of the jhp0917 leading to a continuous gene of 1839 bp (Ahmed et al., 2012). *dupA* was first revealed in 2005 to be a gene connected to the onset of duodenal ulcer and negatively linked to the risk of developing gastric cancer in certain geographical locations. Work done by Lu and co (Lu et al., 2005) has shown a significant relation between *dupA* and duodenal ulceration, and a negative association with gastric cancer, in subjects from Japan, South Korea, and Colombia. This linkage is particularly so in strains from South America and East Asia (Alam et al., 2020). The research also demonstrated a direct link of *dupA* with increased IL-8 secretion (Lu et al., 2005). There have been several reports to support the findings of Lu et al including investigations conducted in North India, Brazil, and China (Alam et al., 2020). However, studies from other parts of China and Brasil (São Paulo), North America, Belgium, and South Africa found no association between *dupA* and duodenal ulcer in their respective populations. Since its identification, *dupA* has become an interesting gene in terms of clarity in its actual role in disease progression thanks to the discrepancy

in reports from different parts of the world on the role in disease progression (Alam et al., 2020).

The worldwide prevalence of the *dupA* gene has been estimated at approximately 45% (64% in Western countries and 31% in the Asian population) (Alam et al., 2020; Karbalaei et al., 2021). Based on the results of several meta-regression analysis, the presence of *dupA* alone is not primarily indicative of disease outcome risk in *H. pylori* infection. Other factors may play key role in the ultimate indication, such as the presence of other highly toxic bacterial factors. The distribution of variability in clinical evidence is based on differences in geographic regions, *H. pylori* plasticity zone distribution and methods used to identify and analyse it. Confusing results regarding the relationship between *dupA* and gastric ulcer and gastric cancer risk are evident in many studies. Some studies have found associations between *dupA* and clinical outcomes, but several subsequent studies have failed to replicate these results. A meta-analysis was conducted to determine the relationship between *dupA* gene and clinical outcome. It was revealed that 29 Asian studies on *dupA*-positive subjects had a significant association (OR: 1.94; 95%CI: 1.48–2.55; I 2 : 68.64; Q-value: 89.28; p value: 0.01) with PUD. Subgroup analysis revealed higher odds ratios (OR) for Asian countries (OR 1.57, 95%CI: 1.19-2.06) compared to Western countries (OR 1.09, 95%CI: 0.73-1.62). This raises the possibility that *dupA* is a disease-specific virulence factor, especially in Asia (Shiota et al., 2010). Meta-regression results indicated that the virulence factors *cagA* and *vacA* s1 could have a significant impact on GC development.

Although the presence of *vacA* i1 carries the highest risk of severe clinical condition, the coexistence of the *dupA* gene and vacAs1 contributes to delaying the severity of disease progression by at least 20 years (Paredes-Osses et al., 2017). This was observed in a recent study evaluating the protective effect of the *dupA* gene on severe disease progression in *H. pylori* infected females (OR = 0.05, 95%CI: 0.01-0.42) (Paredes-Osses et al., 2017). *DupA*'s role in the pathogenesis of *H. pylori* infection lies in the activation of mitochondria-dependent apoptotic pathways in host cells, ultimately preventing gastric cell progression.

Induced by contact with epithelium gene A (*iceA1*/*iceA2*)

The *iceA* gene was formerly identified in 1998 during an investigation of genes that were "induced by the contact of *H. pylori* with gastric epithelium" (Peek et al., 1998). Two allelic variants of *iceA* were found, *iceA*1 and *iceA*2. These two are flanked downstream by a CTAG-specific methyltransferase gene called hpy1M, and upstream by the cysE gene, which encodes a protein homologous to the *H. pylori* serine acetyltransferase (Peek et al., 1998). In contrast to the *iceA2* type, the *iceA*1 variant has high homology (66 %) and is upregulated with the *Neisseria lactamica* type III restriction enzymes (nlaIIIR) whenever *H. pylori* gets into contact with host epithelium (Kidd et al., 2001). This *iceA*1-hyp1M linkage forms a system of a restriction-modification unit (Xu et al., 2002). This system is capable of methylating DNA to protect it from recognition and cleavage by restriction enzymes (Proença-Modena et al., 2009b; Yamaoka et al., 1999). *iceA*1 is associated with increased IL-8 levels and PUD (Peek et

al., 1998; van Doorn et al., 1998). In addition, epidemiological studies have revealed a relationship between *iceA* and the development of gastric and/or duodenal ulcers (Kidd et al., 2001). A meta-analysis on western countries has shown an increase in the risk (OR = 1.28) of peptic ulcer in subjects with seropositive *iceA*1 gene (Shiota et al., 2012). Works by other scientists suggest an indication of geographical genetic variability as the main information to be aligned to *iceA* gene but not in relation to specific gastric disease (Caner et al., 2007; Podzorski et al., 2003; Yamaoka et al., 1999).

Diagnosis of *H. pylori* Infection

Invasive investigation tests

Based on certain specific observable features of the gastric mucosa, many efforts have been made towards the evaluation of the accurateness of standard white light endoscopy (WLE) in the identification of *H. pylori* infection. Features observed during endoscopy such as nodularity of the antrum have been linked to a sensitivity and specificity ranges of 39.8 – 96.4 % and 83.6 to 100 % respectively (Dore & Pes, 2021; Loffeld, 1999; Luzza et al., 2001). Other signs of mucosal infections are erosions, erythema, a mosaic appearance with or without hyperemia, absent or thickened folds of rugae, and visible submucosal blood vessels in the gastric mucosa (Bah et al., 1995; Matrakool et al., 2016; Redéen et al., 2003). Further studies report of black spot appearances in the mucosa (Hatano et al., 2018). A research in Japan reports 89 % and 77 % accuracy respectively for the use of nodularity and mucosal swelling in a standard endoscopy to determine a possible bacterial infection and associated

atrophy (Okamura et al., 2018). Since gastritis may occur with or without *H. pylori* infection, a low inter-observer agreement constitutes a major limitation to the translation of gastric mucosal features into diagnosis of infection. The mucosal vasculature should be well highlighted and enhanced imaging techniques such as narrow band imaging (NBI) should be used to make the view as clear as possible. NBI uses blue light from a laser source (415 nm) to highlight the vasculature of the gastric mucosa. This has been found to show distinct patterns of gastric giving a more accurate prediction of *H. pylori* infection (Tongtawee et al., 2015). Using light blue crests or white opaque substances, the conventional NBI magnifying technique gives more than 95 % specificity and sensitivity in detecting intestinal metaplasia (Kanemitsu et al., 2017; Tahara et al., 2017).

Recent developments aim at identifying high-definition endoscopy methods for infection diagnosis and for the detection of gastric lesions. This is expected to help in the realisation of real-time decision-making. Advancements have produced developments like that produced by Nakashima et al. (2018) who developed an approach that uses artificial intelligence. This methodology works by mirroring the brain neural network through the use of BLI-bright and linked colour imaging (LCI). The method proved better in the accuracy and productivity of endoscopic examination with a sensitivity around 96.7% for BLI-bright and LCI (Nakashima et al., 2018). A recent meta-analysis by Bang et al., has revealed that an artificial intelligence algorithm is a promising tool

for accurate prediction of *H. pylori* infection in endoscopic procedures and hence worth evaluation in clinical studies (Bang et al., 2020).

Several diagnostic methods require the use of gastric tissues samples which inadvertently means endoscopy must be performed. Methods such as culture, histology and rapid urease testing are invasive techniques requiring endoscopy and biopsy. The diagnostic method to be employed influences the specimen site and number. Equal numbers of biopsies are taken from the antrum and corpus for histology and culture in *H. pylori* eradicated patients while samples from the distal antrum (2cm from the pylorus) are taken for pre-treatment diagnosis (Pajares-García, 1998). Two or sometimes more of these tests are employed together in diagnosis for higher sensitivity and specificity. For instance, a combination of two methods are used for clinical trials and in the clinical management of patients for improved sensitivity and specificity (Pajares-García, 1998).

Histological methods

Histology is considered one of the most effective ways of infection detection and is primarily about the staining of gastric biopsies (Benoit et al., 2018; Dixon et al., 1996a; Lee & Kim, 2015). Haematoxylin-eosin staining is a common staining technique for *H. pylori* detection and results from this method is about 94 % similar to that of immunohistochemistry in most cases of active chronic gastritis (Benoit et al., 2018). Factors such as site, size and number of biopsies are areas to be considered in ensuring better sensitivity and accuracy in diagnosis (Nakamura, 2001). Biopsies from five different sites is reported to be

ideal for *H. pylori* prime assessment whereas specimens from at least one quadrant in the ulcer edge of the gastric mucosa are recommended for both infection detection and assessment of gastritis (Dixon et al., 1996a). Obtaining samples by following these measures reduces error associated with differences in bacteria density at various sites. Over the years, there have been remarkable modifications to existing histology stains. These changes have been reported to have remarkably improved upon results giving considerably higher accuracies and sensitivities (Genta et al., 1994; Vartanian et al., 1998). One such improvement was the simplification of the Giemsa technique into a more time efficient procedure but whose results were similar to the robust traditional one and even more accurate than rapid urease test (Fan et al., 2020). Others include the use of Warthin-Starry stain although which is much more specific although less sensitive (Farouk et al., 2018). This is however, very appreciated in cases where the coccoid forms of the organism is most suspected (Chan et al., 1994). Other stains reported for successful use in *H. pylori* identification include Romanowski, (Zaitoun, 1992) Toluidine blue, (Alturkistani et al., 2016) Gimenez (Suehiro et al., 2019), and the modified McMullen's type which is a modified form of the Gimenez stain as described by McMullen et al (McMullen et al., 1987). Whether low or high bacteria densities, these methods are good percentages of positive identification with good sensitivities as well (Laine et al., 1997). Immunohistochemical determinations are very sensitive and specific and hence a repeat of endoscopy in patients who test negative even though they have a high index of suspicion (Park & Koo, 2014).

Also, the proposed staging systems by Capelle et al. (Capelle et al., 2010), and Rugge et al. (Rugge & Genta, 2005), that is, Operative Link on Gastric Intestinal Metaplasia (OLGIM) and Operating Link for Gastritis Assessment (OLGA) respectively, are recommended for histopathologic examinations. The OLGA system has been applied among a Chilean population in the score of lesions associated with the corpus and antrum for evaluation of the risk of early gastric cancer or neoplastic evolution (Bellolio et al., 2019). Generally, in clinical practice, sampling error is cited as a cause of inaccurate histological diagnosis, so it recommended that additional specimens be taken from the lesser curvature and/or the gastric body to increase the diagnostic accuracy (Metz et al., 1998; Westblom & Bhatt, 1999).

Culture approach

The culture of *H. pylori* is typically difficult because it is an obligatory microaerophilic organism that requires special media and culture conditions for growth. Again, the usual spiral shape of the organism changes to coccoid in adverse conditions and loses its culturability on media (Owen, 1995). These challenges notwithstanding, the culturing of this fastidious organism remains an important method since it represents one of the most specific methods in the identification and diagnosis of *H. pylori* infection (Metz et al., 1998). Several factors contribute to the sensitivity of the culture approach of this very demanding bacterium because of which considerations for samples taking, transport of biopsies to the laboratory, media and incubations conditions are paramount (Thomas et al., 1992; Westblom & Bhatt, 1999). The successful

growth is also facilitated by the use of suitable augmentation media together with specialised antibiotic supplements for a reduced oxygen environment are essential for successful growth of the organism. The organism have been isolated successfully with considerable recovery rates from sources such as vomitus, mucosal brushings, gastric biopsies or fluids, luminal aspirates, saliva and dental plaques have been documented (Ferguson et al., 1993; Madinier et al., 1997; Oshowo et al., 1998; Shames et al., 1989; Thomas et al., 1992). The most widely utilised specimen with proven reliability indices is biopsy samples obtained from the corpus and/or antrum of the gastric mucosa (Genta & Graham, 1994; Karnes Jr et al., 1991; Penner, 1991). Biopsies from outside the gastric mucosa are poor and usually not easy to reproduce (Bernander, 1993; Desai, 1991). Recovery is also affected by the presence of other microorganisms of the oropharyngeal environment (Glupczynski, 1998). Therefore, biopsies are usually rinsed or minced in 0.9 % saline to improve isolation rates of cultures (Jonkers et al., 1996).

Biopsies for culture are taken by Specialists at various endoscopy units in health facilities for onward transportation to research facilities for further analysis. The type of transport medium used to convey specimens from collection site to the laboratory is key for the successful culturing of *H. pylori* (Glupczynski, 1998). Since the viability of the bacterium reduces with increasing atmospheric oxygen, it is important that the samples collected into appropriate tubes are kept on ice after collection and processed for culturing as quickly as possible (Owen, 1995; Soltesz et al., 1992; Veenendaal et al., 1993). Where storage is expected

to last more than 24 hours, storage medium of cysteine with 2 % glycerol stored at -70^{o}C (Owen, 1995) or sterile Brain Heart Infusion Broth (BHI) containing 0.25 % yeast extract, 5% horse serum, nalidixic acid (20 mg/L), vancomycin (6 mg/L), amphotericin B (4 mg/L), have been found to produce higher culture recovery rates (Siu et al., 1998). A specialised medium that could preserve the characteristics of *H. pylori* isolates at 4 ^{o}C and lasting up to a year has been developed by Italian researchers. The difference in colony count was not statistically significant (Cellini et al., 2014). Even though some investigators have reported the successful use of normal saline as a transport medium for gastric biopsies, (Cellini et al., 1996; Penner, 1991; Veenendaal et al., 1993) the ultimate goal has been to provide temporal nourishment for the organism and prevent the drying up of the specimen. Another important factor is also to, as much as possible, minimise the exposure of the organism to atmospheric oxygen which may cause their death.

In the actual culture procedure, researchers have come out with different culture medium and various supplementations for a successful isolation. The recipes for media include adding Skirrow's supplement (SR69) and 7 % Horse blood to Brain Heart Infusion (BHI) or supplementation of Belo Horizon medium with 2,3,5-triphenyltetrazolium chloride (TTC). Distinctly sparkling isolates are observed when the medium is supplemented with TTC which helps in the easy identification and recognition of *H. pylori* colonies (Adinortey et al., 2018). Sensitivities that go as high as 90 % have been achieved with BHI medium containing antibiotic supplementations and red blood cells (Dent & McNulty,

1988; Jiang & Doyle, 2000). With culture as the identification approach, investigators have the added chance of assessing the antibiotic susceptibility to determine the most efficient treatment regimen (Deltenre et al., 1989). Meanwhile, culture procedures are very involving with a wide variation in the rate of recovery coupled with high cost and time consuming factors.

Rapid urease tests

Clinical diagnosis usually requires a quick procedure that is also not so expensive and the rapid urease test (RUT) for *H. pylori* is a good example. Sometime in 1985, Owen and co recounted how *H. pylori* was capable of inducing rapid urease hydrolysis, an activity that was different from many bacteria (Owen, 1995). Many kits have been developed for diagnostic purposes based on this simple biochemical urease reaction. The procedure however uses a gastric biopsy which implies the use of invasive techniques to obtain the specimen. The specimen is applied to urea substrate and a change in colour is observed as a result of the release of ammonia by the action of the bacterium. A pH indicator is embedded in the kit and with the help of a bacteriostatic agent, the released ammonia increases the pH causing a change in colour from yellow to red (Westblom & Bhatt, 1999). Manufacturer recommendations indicate the time after which results of rapid urease test may be deemed unreliable. Reports indicate that specificity falls to about 68 % and the likelihood of recording false positive results are raised when results are read after more than 1 hour (Yousfi et al., 1997). RUT results can be as sensitive enough to be comparable to the results of culture irrespective of testing time but not as the method by histology

if the specimen is from patients who have had treatment within some few months back (Murata et al., 1999). Thus, the test kit demonstrates fairly accurate results when the test is performed after several months of therapy making it preferable in confirming successful treatment (Murata et al., 1999).

Molecular detection

Molecular tests using Polymerase Chain Reaction (PCR) are very important for early infection detection and for confirmatory purposes where precise diagnosis is essential (Ashton-Key et al., 1996). Stool and saliva specimens have recorded detection of infection using molecular methods but the disadvantage is in the low numbers and therefore not very ideal. Recently, improvements in testing for the presence of infection in these specimens have been found in the application of CRISPR-Cas12a technology. The method is simple, rapid, and inexpensive but with poor levels of sensitivity and specificity (Qiu et al., 2021). To improve upon the sensitivity and specificity for this non-invasive molecular method, Bangpanwinom and others reported a procedure that was 100 percentage more sensitive than the conventional PCR method (Bangpanwimon et al., 2021). The method employs a colorimetric magneto loop-mediated isothermal amplification (CM-LAMP) assay on spiked saliva samples. Isothermal applications have been combined with a sensitive DNA sensor for an improved sensitivity and stability using a 17 nt target DNA primer specific for *H. pylori* (Song et al., 2021). The PCR approach is reliable and accurate and the findings are comparable to the combined results of detection by culture and histology (Lage et al., 1995; Lin et al., 1996). Earlier, an approach that relied on

small RNA molecules called Nickel-regulated small RNA (NiKs) was found to be associated with bacterial signalling pathways and applicable in detection (Eisenbart et al., 2020). These small RNAs were found to be related to the regulation of most of the virulence factors of *H. pylori* infection. Bacterial virulence factors allow the bacteria to persist in the harsh environmental conditions of the stomach leading to disease development (Deng et al., 2020; Eisenbart et al., 2020).

Non-invasive investigations

Rapid urine antibody tests

Urine specimen for infection detection is a non-invasive procedure that can be developed to give better accuracy and sensitivity than it does currently. Against this backdrop, Japanese researchers have developed a rapid test kit for urine *H. pylori* IgG antibody (RAPIRUN) that uses ELISA or immunochromatographic approach (Alemohammad et al., 1993; Graham & Reddy, 2001; Katsuragi et al., 1998). Some studies have concluded that the urine-HpAb rapid test has lower sensitivity when compared to non-invasive methods such as UBT and stool antigen test (SAT) (Okuda et al., 2013; Okuda et al., 2017) as well as invasive methods like culture and histopathology (Aumpan et al., 2019). This finding is particularly prominent in children below 10 years (Okuda et al., 2013). On the contrary, studies in Vietnamese, (Graham & Reddy, 2001; Nguyen et al., 2010) Japanese, (Kato et al., 2000; Mabe et al., 2017; Nguyen et al., 2010) and Indonesian (Syam et al., 2015) populations have recorded high specificity and sensitivity values for the determination of *H. pylori* antibodies in urine samples.

In fact, other results found higher accuracy and reliability results of rapid urine test in comparison to culture, RUT, and/or histopathology (Aumpan et al., 2019; Mabe et al., 2017; Miwa et al., 2001; Syam et al., 2015; Wong et al., 2002).

Serological tests

For screening exercises, serologic testing is an important technique of *H. pylori* detection. It is a good alternative for patients who do not necessarily need an immediate endoscopy (Cutler & Prasad, 1996). It is also useful in the categorization of infected individuals into high cancer risk patients. It is however not a good option for therapeutic follow-ups (Makristathis et al., 2019). In general, serology is not ideal for confirmation of effective treatment or complete infection eradication. It is limited to initial diagnosis due to the slow rate of decline in antibody levels after eradication of infection. Antibody levels normally reduce to about 50 % within 6-12 months of eradication. However, lots of patients will still show positive serological tests even if treatment and infection eradication has lasted for more than a year (Cutler & Prasad, 1996; Westblom & Bhatt, 1999). The results of the serological test are obtained within 5 – 10 minutes and hence considered the alternative to qualitative determination (Westblom & Bhatt, 1999). In a recent study into the serum antibody response to immunodominant recombinant antigens among ELISA-positive participants, three antigens (*CagA*, HP0175, and Tip- α) were found to be instrumental in distinguishing between past and current *H. pylori* infection. Here, it was discovered that subjects showing antibody to at least one of the antigens had up

to 5.4 times a bigger risk of having a recent infection as opposed to those with triple negatives (Shafaie et al., 2018).

Stool antigen test

Two recent enzyme immunoassays (EIA) for *H. pylori* antigen in stool have been developed. They are the *H. pylori* QUIK CHEK™ and *H. pylori* CHEK™ assays. These methods target *H. pylori* flagellar protein (flagellin) unlike the reliance on urease activity by other conventional detection assays. Sensitivities of 92 % and 91 % and specificities of 91 % and 100 % have been obtained for the QUIK CHECK and CHEK respectively (Halland et al., 2021). Quick Chaser *H. pylori*® (QCP, Misuho Medy, Tosu, Japan) is another example of flagellin-detecting test kit whose results are comparable in efficiency to the catalase-based commercially available Testmate Rapid *Pylori* Antigen® designed by Wakamoto Ph., Tokyo, Japan (Kakiuchi et al., 2019). In order to improve upon this, stool samples can be spiked to enhance the sensitivity and selectivity (Jain et al., 2020). The approach uses *H. pylori* immunosensors of high performance to appraise the antigen-antibody interaction without unsettling conjugate biomarkers. Faecal antigen detection and UBT are acclaimed non-invasive ways for verification of *H. pylori* infection in children because serologic tests are less consistent and are suitable only for screening such a population (Ni et al., 2000; van Doorn et al., 2001).

Other recent stool antigen tests for *H. pylori* detection include the use of bioluminescent enzyme immunoassays (Kakiuchi et al., 2022) and fluorescent aptasensor (Zou et al., 2022) for ultrasensitive detection. The latter has been

modified with the inclusion of gold nanostructures integrated on hollow carbon N-doped capsules.(Roushani et al., 2022) These methods are regarded as reliable and futuristic procedures showing lots of promising advantages over traditional ones.

Urea breath tests

The Urea Breath Test (UBT) is a further non-invasive technique that exhibits encouraging outcomes. It utilises the ability of *H. pylori* to produce the enzymes urease. The approach entails giving an oral dose of urea meal that has been 13C- or 14C-labeled, followed by the assessment of expired breadth during a 2-hour period (Debognie et al., 1991; Graham et al., 1987; Savarino et al., 1999; Toyama et al., 1999). In a person with *H. pylori* infection, urea is converted into ammonia and bicarbonate, with the latter being transported to the lung. The labelled carbon (IV) oxide expired from the lungs can then be measured. Bacterial burden in the gastric space can be determined when ^{14}C is used because quantification of ^{14}C is easily done by the use of a scintillating counter. The advantage in the use of ^{13}C label is in its non radioactiveness but the counting will require a more costly and complicated gas isotope ratio mass spectrometer (Graham et al., 1987; Savarino et al., 1999). Results from UBT are sensitive and highly specific but can be influenced by the other urease producing organisms especially for patients without acid secretions (Breslin & O'morain, 1997; Westblom & Bhatt, 1999). UBT is the gold standard for testing in asymptomatic patients after treatment and hence very useful in determining the success of infection eradication (Metz et al., 1998). It is very sensitive in

identifying traces of *H. pylori* and as such widely used in various epidemiological and treatment studies (Deguchi et al., 2020; Makristathis et al., 2019). According to recent studies comparing various methods, UBT had a higher diagnosis accuracy than other non-invasive diagnostic tests in patients who had never undergone a gastrectomy, hadn't taken antibiotics, or used proton pump inhibitors (PPIs) (Best et al., 2018).

Therapeutic Consideration against *H. pylori*

Effective bactericidal effects of drugs agents against *H. pylori* infection are achieved when the gastric mucosal pH is increased using antisecretory agents. When antibiotics are used alone in the infection treatment, the ease to develop resistance increases. Herbal preparations and probiotics that are used as complementary remedies do not have a clear mechanism of action making antibiotics largely relied on (Yang, 2016). The chances of antibiotic resistance development is largely reduced when different groups of antibiotics are combined in a single therapy (Pohl et al., 2019). That is, to effectively treat *H. pylori* and reduce the chances of antibiotic resistance, the recommended treatment type will be the combination of several antibiotics (Pohl et al., 2019). *H. pylori* is treated with a variety of proton pump inhibitors, antimicrobials, and antisecretory medicines. Amoxicillin, metronidazole, levofloxacin, tetracycline, clarithromycin, rifabutin, and substances containing bismuth are among them (Fan et al., 2020; Flores-Treviño et al., 2018). The treatment takes into consideration previous antibiotic exposure before administration of

interventions. This brings about the lines of therapy for treatment, grouped into first-line, second-line and third-line therapy.

First-line therapy

Consistent with several international treatment guidelines, first treatment option for *H. pylori* is a triple clarithromycin-based antibiotic therapy administered to persons living in areas that are low in clarithromycin resistance for a period of 7–14 days. This regimen is to be restricted to patients who have had no prior exposure to macrolides (Mladenova, 2021). It may comprise of clarithromycin together with either amoxicillin or metronidazole, and a Proton Pump Inhibitor (PPI) or ranitidine bismuth citrate (Suzuki & Mori, 2018; Suzuki et al., 2010). Available data shows the most preferred is the bismuth quadruple therapy or the combinations of PPI, amoxicillin, clarithromycin and nitroimidazole popularly called concomitant non-bismuth quadruple therapy (Mladenova, 2021). These combinations have proven to be an effective way of overcoming drug resistance. After a failed first-line treatment attempt, a second attempt is initiated. This second attempt should avoid any antibiotic that was used in earlier treatment. The best approach will be to experimentally carry out an individual antibiotic resistance and sensitivity test in order to identify which antibiotics will be effective. Again, it is recommended that a symbiotic be added to the standard antibiotic regimen to facilitate the eradication rate and also reduce adverse effects of the treatment action (Gunaratne et al., 2021). First-line rescue treatment should avoid clarithromycin which was included in the first-line treatment and replaced with levofloxacin or bismuth salts schemes (Graham &

Fischbach, 2010). Bismuth quadruple therapies involving levofloxacin have gained wide acceptance as an effective therapy in most countries and highly recommended after failure of quadriceps containing clarithromycin.(Chey et al., 2018; Pellicano et al., 2018).

As resistance to antibiotics keeps increasing, modifications are done to rescue treatment. Antibiotics have been studied comprehensively and show increasing levofloxacin and clarithromycin resistance in several areas (Gong et al., 2020; Shrivastava et al., 2018). Currently, in Japan, a competing potassium acid blocker called vonoprazan, has been used on difficult-to-treat groups of patients instead of PPI (Mladenova, 2021). Vonoprazan has an inhibitory action on the secretion of acid in the stomach and can be combined with a single antibiotic to treat the infection. Research has shown that this combination is 91 % effective in treating patients with previous eradication failure and up to 100 % in patients who take them as first line treatment (Gunaratne et al., 2021). In addition to this, areas with increased resistance to clarithromycin and/or metronidazole are recommended to take bismuth or non-bismuth quadruple therapy for 2 weeks as their first treatment (Suzuki et al., 2019).

Second-line treatment

Whenever a first-line treatment fails, it basically means a failure in an antibiotic due to the development of microbial resistance and new methods are needed. A first-line failure means a second-line therapy is required. This usually involves a triple/quadruple fluoroquinolone–amoxicillin therapy or a quadruple bismuth therapy. Their efficacies are similar though the first has lesser side effects than

the second. The Maastricht V/Florence Consensus Report supports second-line therapy as an eradication remedy for first line failure and recommends quadruple bismuth therapy (Malfertheiner et al., 2017). Analysis of recommendations from various guidelines suggests a triple therapy treatment based on rifabutin or high-dose amoxicillin plus PPI, or a triple levofloxacin therapy for infection eradication after failure of first-line therapy (Fallone et al., 2019; Puig et al., 2016). For instance, it has been discovered that tetracycline-levofloxacin therapy is efficient when used for up to 10 days as a second line treatment (Lin & Hsu, 2018; Nguyen et al., 2019). Antibiotics that were utilised in the first treatment are to be avoided in eradication therapies.

Third-line (and further) treatment

Antimicrobial susceptibility testing on a culture of *H. pylori* should be performed after failure in a second-line treatment. Afterwards, a good account of the better choice of antibiotic may be obtained to continue with treatment using the right antibiotic option (Malfertheiner et al., 2017). By the time treatment reaches this point, antibiotic resistance may have shown wide coverage in terms of the number and types of antibiotics making the cure rate in third-line therapy only moderate (Davies et al., 1994). The levels of drugs administered in this line are those of high concentrations which may have adverse effects on the body. Prolonged PPI usage for instance, is known to inhibit micronutrient absorption and reduce the benefits derived from them (Haastrup et al., 2018). Increased risk of hypomagnesemia and fractures as a result of long-term PPI use have been reported by the United States Food and

Drug Administration (Öztekin et al., 2021). Patients with two previous therapy failures are given high doses of metronidazole (500 mg to be taken three times daily for 14 days), amoxicillin (1 g to be taken three times daily), and esomeprazole (40 mg taken two times daily) as eradication therapies (Puig et al., 2016).

H. pylori treatment rates have been improved by the use of probiotics. Probiotics are also very useful in their application as measures to reduce side effects after long term antibiotic use. In an experiment involving 159 patients in receipt of sequential *H. pylori* eradication therapy, higher clearance rates were obtained for a 14 day adjuvant application of probiotics. In addition to the higher eradication rate, treatment with probiotics produced less side effects and reduced diarrhoea which mostly results in treatment discontinuation (Çekin et al., 2017).

Relapse and reinfection

There are two types of *H. pylori* infection recurrence: reinfection and recrudescence (Sjomina et al., 2018). Recrudescence is the re-emergence of the initial infection following an initial false-negative test result and an eradication test result (Sarem & Corti, 2016). There is where *H. pylori* is realised to have remained dormant (example *H. pylori* coccoid forms) or has been tucked away in the deepest part of the stomach or the gastric epithelial metaplasia of the duodenum. It finally returns, reproduces, and is discovered after some time. Reinfection is therefore caused by an infection with a new strain or a strain that

is identical to the original strain of *H. pylori* Recrudescence strain is the initial infectious strain.

The complete treatment of diseases such as peptic ulcer, gastritis, and the early prevention of gastric cancer cannot be wholly achieved while *H. pylori* infection persists. Eradication challenges consequently constitute a major task in dealing with gastric related clinical conditions (Diaconu et al., 2017; Kim et al., 2014). Between 2000 and 2010, Zhao et al. (2021) reported an *H. pylori* eradication rates of between 80%–90% in general and 40%-60% for persons having drug resistant strains (Cortés et al., 2021; Glupczynski et al., 2001; Liu & Liu, 2019). For areas with a high number of infected individuals, the chances of reinfection is also high. The presence of family members with *H. pylori* infection, deprived state of living conditions, and health status are major examples. Patients who are treated have a greater chance of becoming infected by a strain that is homologous to the previous infection or a totally new strain. Also, when treatment procedures are ineffective or incomplete, recrudescence may occur (Gisbert et al., 1998; Kim et al., 2014; Sun & Zhang, 2019). Reports of high recurrence rates are not restricted to only one form of treatment regimen. There are results showing how varying degrees of recurrence have been found with both triple therapies (14 %) and quadruple therapies (12 %) (Zhao et al., 2021). A worrying situation is recognized in the trend observed for treatment follow-ups where an increasing rate of 4 %, 6 %, 8 % and 12 % was realised for the first, second, third, and fourth or longer follow-ups respectively. Recurrence is lower in patients exposed to quadruple therapies than those who took triple

therapies. It must be noted however that *H. pylori* resistance to antibiotics varies from one geographic location to another, a situation that in turn varies the eradication rate (Hooi et al., 2017). Even in the same country where treatment schemes may be similar, the rate may vary from one region of the country to another. This implies, the choice of treatment regimen must be based on local epidemiological surveys (Dang & Graham, 2017; Suzuki & Mori, 2018).

In order to accurately predict reinfection or relapse and match the kind of relapse to therapy and follow-ups, genotypic investigation is crucial (Raymond et al., 2016). In order to determine if an *H. pylori* recurrence is a recrudescence or reinfection case, genotyping techniques are used to compare the *H. pylori* strain before and after recurrence. Relapse frame is relatively shorter compared to reinfection, after which recurrence of *H. pylori*-related diseases take effect. It is very rare for a patient to be re-infected if the eradication therapy was an effective one (Stefano et al., 2018). Even so, the factors for *H. pylori* relapse require extra study (Sun & Zhang, 2019).

Vaccination

In developing countries where prevalence is high, a radical approach to the reduction in burden of infection would be to develop a vaccine that will become part of the mandatory national vaccination programs for children. This is expected to reduce the early acquisition of the disease and hence a good fight for later gastric disease developments. For an effective vaccine, there is the need to have a better understanding of the complexity of host immune response to *H. pylori* infection as well as the immune mechanisms relating to its genetic

diversity (Mladenova, 2021). *H. pylori* is known to persist due to this diversity and a prophylactic vaccine will have to wait for a while if this knowledge isn't well known (Abadi, 2016; Stubljar et al., 2018). In view of these, *H. pylori* is yet to have an effective immunogenic vaccine and more investment is needed to up research in this direction (Ribaldone et al., 2019; Sutton & Boag, 2019). Some levels of progress have been seen in mouse models where protective immune responses by antigens or epitopes of *H. pylori* together with their adjuvants have been recorded (Mladenova, 2021). Again, work by Zeng et al., reports of trials in Chinese children with promising results (Zeng et al., 2015).

Immunogenic virulence factors have been the selected target proteins for an *H. pylori* vaccine. Once the bacterium's virulence factors are neutralised, its infection will ordinarily be impaired. Such immunogenetic factors include the target of cytotoxin associated gene A (*cagA*) (Takahashi-Kanemitsu et al., 2020) and vacuolating cytotoxin A (*VacA*) (Liu et al., 2004; Moyat & Velin, 2016). The challenge with these targets are in their high genetic variability coupled with the absence of *cagA* in some strains of the bacterium (Gressmann et al., 2005; Linz & Schuster, 2007). If a vaccine which covers the diverse immunogenicity were to be produced, it will prevent this infectious microorganism from evading the host immune system. A number of researchers have therefore targeted proteins based on various combinations of virulence factors as the basis for the proposal of vaccine candidates. This includes a target of *VacA*, *CagA*, and neutrophil-activating protein (NAP) (Malfertheiner et al., 2008) or by *CagA*, *VacA*, and urease subunit B (*UreB*) (Liu et al., 2011), or by

UreB, NAP, adhesin A (HpaA) and heat shock protein 60 (HSP60) (Guo et al., 2017). Others have used *UreB*, Flagellin A (FlaA), *cagA*, *VacA*, , and Adhesin-binding fucosylated histo-blood group antigen (BabA) (Calado, 2022). In the development of vaccines of this nature, the greatest test lies in the complexity of the production process, time and costs. Epitope-based vaccines remain the most probable candidate for this situation where the epitopes are presented as a set of peptides or included in a polypeptide (Nandy et al., 2018; Skwarczynski & Toth, 2016; Yang et al., 2015).

Antibiotic Resistance

H. pylori antibiotic resistance has seen a worldwide drastic increase in occurrence and there seems to be an increase also in multi-drug resistance (MDR). MDR is increasingly becoming an obstacle to the complete eradication of the infection. There has been triple, quadruple and quintuple MDR identified at certain places. This suggests the need for improved measures on the administration of antibiotics to *H. pylori* infected individuals. *H. pylori* drug resistance mechanisms are basically due to point mutations sited on the chromosome (Gerrits et al., 2006; Tuan et al., 2019). For instance, several point mutations in the *rrl* gene that encodes two (2) *23S* rRNA nucleotides: A2142C, A2143C, T2182C, A2142G, A2143G, etc are mainly responsible for clarithromycin resistance in *H. pylori infection* (Seo et al., 2019; Van den Poel et al., 2021; Wang et al., 2020). *H. pylori* develops fluoroquinolone resistance due to frequent mutations at codons 87 and 91 in the gyrA (gyrase A) gene's quinolone resistance-determining region (Arslan et al., 2017). Resistance-

related mutation sites may be different for different geographical locations (Salehi et al., 2019; Shetty et al., 2019) and these differences are very relevant when it comes to ensuring no false negatives results are reported. Confirming *H. pylori*'s resistance mutation sites is crucial for generating reliable data that can be used to further create significant mutation sites connected to antibiotic genes.

Various investigations have shown that drug efficacy is directly linked to bacterial resistant gene point mutations. Genes encoding the targets for antibiotics become 'non recognizable' to their respective antibiotics. Mutations in such genes that encode targets for drugs such as clarithromycin and levofloxacin are consistent with results of clinical efficacy of *H. pylori* infection and drug sensitivity tests (Kageyama et al., 2019; Lauener et al., 2019; Salehi et al., 2019; Tuan et al., 2019; Zhang et al., 2019). This means genotypic antibiotic susceptibility analysis can be applied in clinical therapeutic guidance. Demonstration of phenotypic drug susceptibility or resistance have continuously been found to be consistent with mutations at V domain of *23S* rRNA for clarithromycin resistance, gene mutations at *gyrA* for levofloxacin, and gene mutations at *pbp1* (penicillin binding protein 1) for amoxicillin resistance (Tuan et al., 2019). Conversely, the gene mutations of *rdxA* (oxygen insensitive NADPH nitroreductase) for metronidazole resistance and *tet-1* for tetracycline resistance have not been wholly consistent with the phenotypic resistance (Tuan et al., 2019).

Where there is a clear indication for inference to gene analysis for therapeutic purposes, treatment plans have been relatively more successful. Knowing the resistance or susceptibility of subjects at the genotypic level means targeting individualised treatment which yields better drug efficacy results According to research done in 2021 by Cui et al. (2021), in a case of clarithromycin genotypic resistance, a rate of 57% eradication was achieved for the gene-resistant group, which was significantly lower than the 87.8% rate achieved for the gene-sensitive group (87.8%). This difference of over 30 % in eradication rate of clarithromycin related treatment is an indication that personalised therapeutic measures guided by genotypic antibiotic susceptibility can efficiently improve eradication rate (Cui et al., 2021). Satisfactory and clinical effectiveness without noticeable adverse reactions guided by genotypic analysis of antibiotic susceptibility has been achieved in other studies (Arenas et al., 2019; Gao et al., 2020).

CHAPTER THREE

RESEARCH METHODS

The study looked at generating data on the prevalence of *H. pylori* infection in the Central Region of Ghana and also provide information on the state of antibiotic resistance regarding *H. pylori* infection. It also assesses the distribution of virulence genes in gastroduodenal patients visiting reference laboratories for various gastroscopies in the Central region of Ghana. This chapter examines the research methodology employed in obtaining data for the study. It describes sampling procedures, ethical considerations, biopsy processing procedures and analysis. The chapter also looks at bacterial isolation and identification steps, antibiotic susceptibility testing and DNA extraction techniques used. The chapter looks at how various genes were identified by PCR and electrophoresis as well as methods employed in investigation of gene sequences for mutations and subsequent prediction of secondary and tertiary structures.

Ethical Considerations, Subject Enrolment and Consent Procedure for Biopsy Acquisition

All endoscopy procedures were performed by an experienced Gastroenterologist. Sample acquisition and retrieval of secondary data begun after approval by review committee of the Ethical Review Board, University of Cape Coast, Ghana. The ethical clearance number UCCIRB/CANS/2019/05 was assigned to the study. All adult subjects who were referred for diagnostic upper gastrointestinal endoscopy were informed about the study. Details of the

study were described to them in English and also translated into local language which was well understood by all who had challenges with English. Detailed information was given about the study after their usual early morning patient-nurse education/interaction session. The nature of information required on questionnaires were explained and patients were asked if they would consent to the taking of additional biopsies required for the purpose of the research (to be taken from the gastric antrum).

For subjects below 18 years accompanied by their relatives and ready to partake in the study, such adult relatives consented for the 'minors'. Afterwards, a signed consent form was obtained from all those interested. It was clearly stated to subjects that their participation in the study wasn't going to attract any compensation. All those who declined to participate went on with the planned procedure but with no additional biopsies taken from them.

Acquisition and Processing of Secondary Data for the Determination of Dynamics of *H. pylori* Infection.

In order to obtain data that will provide better and clearer picture of the infection dynamics compared to earlier studies in some parts of Ghana, a larger sample size was sort for. Data for determination of dynamics of prevalence of *H. pylori* infection were obtained as a secondary information consisting of past patient endoscopy records from the year 2012 to 2019. The consent of Clinical Directors of the two referral centres included in the study were obtained before accessing the database of their respective facilities. Confidentiality and security of records were assured and clearly explained in the ethical considerations

submitted and approved by ethical review board of the University of Cape Coast.

Exclusion-Inclusion Criteria and *H. pylori* Identification of Secondary Data

All patient reports of complete diagnostic esophagogastroduodenoscopy were included. Incomplete reports and colonoscopies were excluded. For patients who had undergone two or more esophagogastroduodenoscopies, only the report of the first procedure was included. Records without urease test results for the presence of *H. pylori* and those with incomplete patient demographic data were excluded. In all, a total of 2145 endoscopies were available in the database of which 1767 were selected after applying the inclusion-exclusion criteria. For all records, *H. pylori* was stated to have been identified by the rapid urease test (HelicotecUT®Plus, Strong Biotech Corp-Taiwan).

Subject Inclusion and Exclusion Criteria for Collection of Gastric Biopsies

Purposive sampling technique was used to obtain data. All patients referred for diagnostic esophagogastroduodenoscopy at two medical centres that provide endoscopy services were considered eligible for inclusion. Gastric biopsy samples were obtained from the endoscopy centres between October 2019 and August 2021. The two centres are both located in the Cape Coast South Metropolis of the Central region of Ghana. These centres provide healthcare to the people of Cape Coast and receive requests for endoscopy from facilities

throughout the Central Region and sometimes the Western Region. These were the two main referral centres for endoscopy in the area.

Exclusion criteria

Any contraindication to the taking of a biopsy specimen such as those of severe esophago-gastroduodenal disease including suspected advanced gastric cancer patients, persons with a history of prolonged bleeding or serious organ dysfunction (e.g kidney, liver, heart) were excluded from the research. Patients whom the gastroenterologist observed a gastric stricture resulting in some form of challenge to obtaining a clearer endoscopic examination were also excluded. Furthermore, in situations where the endoscopist felt the procedure was being prolonged because of a special situation that requires wider examination such that trying to take the research biopsies wasn't going to be advisable, such subjects were also excluded.

Collection and Transportation of Gastric Biopsies

The endoscopic performance was a component of a normal inquiry into the underlying cause of their ailment. After each patient gave their agreement to take part in the study, two (2) antral stomach biopsies were taken from each patient. The biopsies were placed in separate tubes containing brain heart infusion (BHI) broth supplemented with glycerol and kept on ice. All samples were maintained in ice chests and delivered right away to the Molecular Biology and Biotechnology lab for culture and/or DNA extraction. A total of 169 gastric biopsies (two from each patient to make a grand total of 338) were obtained purposively for the study.

Preparation of Brain Heart Infusion Broth as Transport Medium

BHI broth was used as a carrier medium to transport biopsies. It was prepared by measuring the following chemical components in varying amounts. It included the measurement of 2.13 g of BHI broth powder, 14.4 mL glycerol, 0.36 mL of 0.2 g/L cysteine powder in 2.5 mL of 1M HCl and the resultant solution adjusted to a pH of 7.40. Aliquots were transferred to cryotubes and sterilized by autoclaving at 121°C for 15 minutes. The prepared transport media were placed in a rack and stored in the refrigerator.

Gastric Biopsy Genomic DNA Extraction Procedure

One of each of the biopsies obtained from the subjects were homogenised using a chilled mortar and pestle following the manufacturer's recommendations for DNA extraction from tissue. The extraction procedure was done using Wizard Genomic DNA purification Kit (Promega Corporation, USA). A total of 169 gastric biopsies from the antrum of dyspeptic patients were taken through the extraction procedure. Briefly, 600µl of chilled Nuclei Lysis Solution was added to the grinded biopsy specimen and thoroughly mixed. The mixture was carefully pipetted into sterile microfuge tubes before placing them in a thermal shaker (VWR Thermal shake lite, China) for incubation at 65^{o}C for a period of 30 minutes. To the nuclear lysate obtained after incubation was added 3µl of RNase Solution, well mixed by repeated inversion of the tubes before another incubation of the resultant mixture was done at 37^{o}C for 30 minutes.

After the samples had cooled to room temperature, 200µl of Protein Precipitation Solution was added. Vigorous vortexing was done for 20 seconds

followed by chilling of samples on ice. At a speed of 15000 g, the samples were placed in a centrifuge (VWR Microstar 17, Germany) and spun for 5mins. The DNA-containing supernatant was poured into a clean microfuge tube that held 600 µL of room temperature iso-propyl alcohol. By repeatedly flipping the tube, the mixtures were thoroughly mixed before centrifuging for 2 minutes at 15000 x g. The visible bulk still in the tube was thrice washed with 70% room temperature ethanol after the supernatant was decanted. To remove the ethanol, the mixture was centrifuged at 15,000 × g and the ethanol carefully aspirated with a micropipette. The tubes were inverted on sterile absorbent paper to drain out the ethanol before complete drying of the DNA was done by keeping the opened tubes in the thermal shaker set to 40^{o}C for about 20minutes. DNA Rehydration Solution (100µL) was added and the DNA rehydrated by incubating the tubes at 65°C for 1 hour. Periodic mixing of the solutions was done by gently tapping the tubes. Extracted genomic DNA was stored at -20°C for later use.

PCR Assays for *H. pylori* Identification, Virulence and Antibiotic Resistance Gene Detections

The extracted DNA samples were removed from the freezer and gently thawed. The presence of DNA was first confirmed by running genomic DNA electrophoresis on a 1.5 % agarose gel. The concentration and purity of the DNA samples were also determined (using SimpliNano manufactured by Biochrom, USA). The confirmed DNA samples were kept on ice for subsequent use including detection and confirmation of *H. pylori* using the *H. pylori* specific

primers Hp23Sr6/r7 and GlmM, identification of virulence factors (*cagE*, *iceA1*, *iceA2*, and *dupA*, *vacA subtypes i1*, *i2*, *s1*, *s2*, *m1*, *m2*), and also for the amplification of antibiotic resistant genes for *H. pylori*. All primers (Merck KGaA, Darmstadt, Germany) were designed based on previously published papers (table 3) with slight modifications of PCR conditions and PCR mixtures. Primers were prepared to a final working concentration of 10µM and the manufacturer's procedure for OneTaq® 2X Master Mix with Standard Buffer (M0482) was strictly followed in the detection of various genes. All reactions were set up on ice and various components of the reaction mixture were added before quickly transferring to the thermal cycler which had been pre-set to the required annealing temperature.

For each microtitre plate, the reaction mixture consisted of 0.5µL each of forward and reverse primers (Merck KGaA, Darmstadt, Germany), 12.5 µL of OneTaq® 2X Master Mix (New England Biolabs Inc, UK), and 6.5µL of molecular grade water. The thawed DNA samples (5µL) were then separately added to their respective tubes before gently mixing the content of the plates. Immediately, the plate was transferred to the thermal cycler (BioRad TT100 thermal cycler, California, USA). Thermal cycling was performed with the first denaturation step set at 94°C for 30 seconds. The 30 cycle steps consisted of denaturation at 94 °C for 30 seconds, annealing at various temperatures for 60 seconds, and a final extension at 68°C for 5 minutes. An infinite hold at 4°C prior to gel electrophoresis to preserve amplified samples. Control set ups included a reaction mixture without DNA as negative control and positive DNA

from previously determined genotypes as positive control. Primer sequences, genes and expected product sizes were obtained from previous studies (table 1).

Amplified products by PCR were electrophoresed on a 2 % agarose. Gels previously stained with ethidium bromide were visualised under UV light. The molecular size marker used in the gels were a 100 bp DNA ladder (Promega Corporation, Madison, USA and Bioneer Cooporation, Korea).

Table 1: List of Primer Sets used for Genotyping *H. pylori* by PCR and their Expected Product Sizes

Gene and Region Amplified	Sequence (5' to 3')	Product Size (bp)	References
Hp23Sr6/r7	CACACAGGTAGATGAGATGAGTA CACA*CAGA*ACCACCGGATCACTA	768	(Suzuki et al., 2013)
Cag E	TTGAAAACTTCAAGGATAGGATAGAGC GCCTAGCGTAATATCACCATTACCC	500	(Mashak et al., 2020)
dupA[a]	ATAGCGATAACCAACAAGAT AAGCTGAAGCGTTTGTAACG	662	(Arachchi et al., 2007; Jung et al., 2012)
iceA1	GTGTTTTTAACCAAAGTATC CTATAGCCAGTTTCTTTGCA	247	(Mashak et al., 2020)
iceA2	GTTGGGTATATCACAATTTAT TTRCCCTATTTTCTAGTAGGT	229 or 334	(Mashak et al., 2020)
glmM	TGCTTGCTTTCTAACACTAACG TTGATGGCGATGCTGATAGG	355	(Mirzaei et al., 2014)
vacA m1	CAA TCT GTC CAA TCA AGC GAG GCG TCT AAA TAA TTC CAA GG	570	(Kishk et al., 2021)
vacA m2	CAA TCT GTC CAA TCA AGC GAG GCG TCT AAA TAA TTC CAA GG	645	(Kishk et al., 2021)
vacA i1	GTT GGG ATT GGG GGA ATG CCG TTA ATT TAA CGC TGT TTG AAG	426	(Khodadadi et al., 2020)

Table 1, continued

vacA i2	GTT GGG ATT GGG GGA ATG CCG GAT CAA CGC TCT GAT TTG A	432	(Khodadadi et al., 2020)
VA1 (S1/S2)	ATGGAAATACAACAAACACAC CTGCTTGAATGCGCCAAAC	259/286	(Kishk et al., 2021; Mashak et al., 2020)
DNA gyarase A (*gyrA*)	AGCTTATTCCATGAGCGTGA TCAGGCCCTTTGACAAATTC	582	(Teh et al., 2014;Wang, 2010; Zhang et al., 2020)
gyrB	CCCTAACGAAGCCAAAATCA GGGCGCAAATAACGATAGAA	465	(Teh et al., 2014; Zhang et al., 2020)
Tetracycline (*tet-1*)	F: TAATTCGATTCTACACGAAG R: CTTCGTGTAGAATCGAATTA	-	(Gerrits et al., 2003)
Pbp1	F: AGCCATTCTTATCGCTC R: CGACTAGCATGGTGATTT	600	(Matteo et al., 2008)
Pbp2	F: AACCGCAAGTTTAGGGTA R: GATCATGCTAGCGTTTAAGT		
Pbp3	F: ACGCGTCTAATGAAGATG R: GTGATGCTTTCAATGAGC		
RdxA	F: GCAGGAGCATCAGATAGTTC R: GGGTGATTTCTTGGTTGC	880	(Pourakbari et al., 2018)

Bacterial Isolation

Preparation of amended brain heart infusion blood agar

By BHI agar was prepared by combining 10.4 g of distilled water with BHI agar powder. This mixture was brought to a boil for a short while before being put into a culture bottle to be sterilized. The medium was autoclaved for 15 minutes at 121°C before being allowed to cool to about 50°C. 0.2 g of tetrazolium salt and 14 mL of laked horse blood were combined with 10 mL of sterile distilled water. In order to prevent the formation of further bacteria and fungi, Skirrow's supplement (SR69) (Oxoid, England) was then added. The slurry was

aseptically put into sterile, dust-free petri dishes after being gently stirred. The agar plates were all left to set up on the work surface.

Inoculation of gastric biopsy and sub-culturing of bacterial isolates

The second set of patient biopsy specimens (total 169) were homogenized in a small quantity of the transport medium and then streaked onto the amended BHI blood agar. All procedures were done under a biological safety cabinet. Inoculated plates were incubated under microaerophilic conditions at 37 ºC for a maximum of 10 days. To confirm the presence of *H. pylori,* routine biochemical assays for bacterial morphology, colony morphology, oxidase, catalase, and urease activities were performed. Colonies were sub-cultured to obtain single colonies and then stored in sterile BHI broth containing 20 % glycerol. Afterwards, they were frozen at − 20 °C until required for further use. All positive cultures were always stored at -20 ºC. In all, fifteen (15) successfully isolated *H. pylori* bacteria were obtained from the 103 *H. pylori* positive biopsy samples.

Identification of *H. pylori* isolates

Typical colony morphology (translucent, convex, round), Gram staining (negative spiral bar), and positive catalase, urease, and oxidase tests are used to identify *H. pylori* cultures. The Wizard Genomic DNA purification Kit (Promega Corporation, USA) was used to extract DNA from bacterial culture in accordance with manufacturer's instructions. The presence of *H. pylori* was again confirmed by the amplifying a region within 23sRNA gene of bacterial DNA specific for *H. pylori* using primer sets Hp23Sr6/r7 (forward 5’ –

CACACAGGTAGATGAGATGAGTA - 3, and reverse 5' - CACA*CAGA*ACCACCGGATCACTA – 3) (Table 1).

Preparation of media for biochemical tests

Christensen urea agar

Two portions of a 1 liter Christensen urea agar medium were made. They were urea concentrate and the basal medium. Basal medium was made by weighing 1.0 g of peptone powder, 2.0 g of potassium dihydrogen phosphate, 5.0 g of sodium chloride (NaCl), 1.0 g of glucose, 15.0 g of agar powder, and 0.012 g of phenol red and dissolving in 900 mL of distilled water. The resultant mixture was vortexed, heated on a hotplate and autoclaved. Preparation of the concentrate was by dissolving 20 g of urea in sterile distilled water (100 mL). The two solutions were thoroughly mixed after which 5 mL was dispensed into sterile round-cap test tubes. They were immediately slanted until set for use.

Normal saline

About 8.5 g of sodium chloride (NaCl) was poured in 1 liter of distilled water and stirred till completely dissolved. Then 5 mL of this solution was dispensed into various screw-cap test tubes and autoclaved.

Biochemical identification *H. pylori* isolates

Preparation of bacterial suspension

Using a loop, 2-3 discrete colonies of a pure culture of *H. pylori* grown on BHI blood agar supplemented with Skirrow's and TTC dye were selected. To create a bacterial suspension, the above mixture was then combined with 5mL of sterile saline and vortexed.

Urease test

Bacterial suspension of each isolate was streaked across the slant of Christensen urea agar. Cultures were monitored for 24-72 hours under 35°C incubation. A magenta color formation shows positive result while no colour change indicated a negative test.

Catalase test

On the glass slide containing the bacterial colonies of each isolate, one drop of a 3% hydrogen peroxide solution was applied. Bubble formation that persisted for close to 10 seconds were deemed a positive indication for the presence of catalase activity by *H. pylori* while no bubble formation was observed for negative test.

Oxidase test

A drop of oxidase test reagent containing 1% tetramethyl-*p*-phenylenediamine dihydrochloride (Becton, Dickinson, USA) was placed on a strip of sterile filter paper. Colony cultures of each isolate were picked, streaked onto filter paper saturated with oxidase reagent, and observed for change in colour within 30 seconds. Positive reactions stained the bacteria purple and colourless or pink indicated negative reactions.

Susceptibility test for *H. pylori* using nalidixic acid and cephalothin antibiotics

A modified BHI blood agar plate was covered with 0.1 mL of *H. pylori* bacterial solution. With the aid of sterile forceps, cephalothin and nalidixic acid discs were applied to the inoculated plate's surface and incubated for 24 hours in a

microaerophilic environment at 35 °C. This was done for all 15 successfully isolated *H. pylori* bacteria isolates.

Gram staining

One-loop full of isolates were smeared on slides and heat-fixed. It was then immersed gently in ammonium oxalate crystal violet solution for a minute. Slides were rinsed under running tap water, after which Gram's iodine was applied for 1 minute before washing again. The smears were destained using absolute ethanol (95%) and washing repeated. Staining was done again but using safranin contrast staining agent for 1 minute 30 seconds performing another washing under running tap water. Slides were dried visualized under simple light microscope (Olympus $CX_{43,}$ UK).

Preservation of *H. pylori*

Primary *H. pylori* plate culture single colonies were suspended in BHI broth containing 20% glycerol. For later usage, this was then placed into cryovials and quickly frozen by putting it in a -80°C freezer.

Antibiogram Test for *H. pylori*

Susceptibility of the 15 successfully isolated *H. pylori* strains to metronidazole (5 µg), amoxicillin (10 µg), levofloxacin, clarithromycin (15 µg), amoxicillin-clavulanic acid (10 µg), tetracycline and ciprofloxacin (5 µg) which are the antibiotics usually indicated for triple therapy were used. Brain heart infusion (BHI) agar containing 7% laked horse blood (Thermofischer Scientific, UK) and *H. pylori* selective supplement was used as previously described. The

turbidity of the prepared bacterial suspension was adjusted to meet the McFarland standard of tube No 3. A sterile glass spreader was used for inoculation onto the medium. Aliquots of standardised bacterial suspension were pipetted onto the media surface and evenly spread on BHI agar plates. All plates were given a 10 to 15 minute drying period. The study's whole collection of 6-mm-diameter Oxoid antibiotic disks was then positioned on the plates. To make sure they completely contacted the agar, they were lightly pushed. To prevent overlapping zones, a space of at least 15 mm had to be maintained from the plate's edge. Plates were kept in microaerophilic conditions overnight at 37^{o}C. Zone diameter breakpoints utilised for clarithromycin, ciprofloxacin, metronidazole, and tetracycline testing were 14 mm, 17 mm, 10 mm, and 16 mm respectively according to Tanih et al. (2010). A breakpoint of 29 mm for levofloxacin was based on a report by Tang et al. (2020).

Sequencing Method

PCR products were first confirmed for the presence of target genes by electrophoresis and submitted for purification and Sanger sequencing by Inqaba Biotec West Africa. Of the 15 *H. pylori* isolates, antibiotic resistance target genes for *gyrA, rdxA* and *pbp1A* were detected at a total of 10, 7 and 10 total genes respectively. These were the samples submitted for sequencing and further investigations. Analysis of DNA sequences and determination of similarities were done with UGENE software using the online BLASTX network available at the National Centre for Biotechnology Information website (NCBI) (http://www.ncbi.nlm.nih.gov). Alignment was done using MUSCLE

and the UGENE software. Mutations in *RdxA* sequences were compared to reference sequences using available online data sets. *H. pylori* strains with accession numbers AF315501.1, AE000511.1 were used for *RdxA* while AY43232.1, AE001439, AB128018, KT966878, and MN538201 were applied to for *pbp*. Analysis of *gyrA* were done in comparison to strains with accession numbers; AE001439, CP002332, and LT837687. Considerations for determination of mechanism of resistance were based on finding non synonymous mutations in *pbp* gene for amoxicillin and to a larger extent amoxicillin-clavulanic acid, *gyrA* gene for levofloxacin and within the *RdxA* gene for metronidazole.

Application of bioinformatics to analysis of genetic antibiotic resistant genotypes were done with assistance from bioinformatics databases such as https://web.expasy.org/translate/ for translation of nucleotides into amino acids sequences, http://bioinf.cs.ucl.ac.uk/psipred/ for prediction of secondary structures, https://yanglab.nankai.edu.cn/trRosetta/output/TR106926/ for tertiary structure prediction and https://bacteria.ensembl.org/index.html for nucleotide position determination. Structures predicted with 'trRosetta' were refined using https://galaxy.seoklab.org/cgi-bin/submit_REFINE.cgi before the wild type and mutant structures were aligned for determination of protein conformational changes using PyMol+Console software. Predicted structures were analysed for percentage amino acids in favoured and allowed regions of the structure as well as number of outliers using Ramachandran plots.

Ramachandran distribution Z scores were recorded for each prediction and these were done using the molprobity.biochem.duke.edu/ online system.

Statistical Analysis

Determination of prevalence was calculated by dividing the total number of persons within the group (eg. members of an age group or gender) by the number of infected participants within that category, multiplied by 100. Analysis of data was done using GraphPad Prism version 8 software. Association between pathological conditions and *H. pylori* infection or virulence factor was evaluated using the Chi-square or Fisher Exact test with Odds ratio. p-value <0.05 were considered statistically significant. Ramachandran plots were used in the analysis of structures of proteins predicted and Z-scores obtained.

CHAPTER FOUR

RESULTS

Prevalence of *H. pylori* Infection Across the Years 2012 to 2019

The overall average prevalence of *H. pylori* infection among males (77.09 %) and females (74.54 %) was determined (Figure 1) from historical patient records. The trend in prevalence for males and females over the years under review was also investigated (Table 2). Across the years, it was found that men had a higher prevalence than women (Table 2), with the exception of 2014 and 2018. All of the analyzed years had an overall infection prevalence of at least 70%, apart from 2012 (47.83%) and 2017 (61.32 %). The year 2018 had the highest rate being 87.50% (Table 2).

Table 2: Prevalence of *H. pylori* Infection among Males and Females from 2012 – 2019

Year	Prevalence (%)		
	Overall	Males	Females
2012	47.83	54.10	40.74
2013	70.92	73.91	68.89
2014	73.07	70.64	74.83
2015	78.01	78.38	77.78
2016	78.62	84.93	73.26
2017	61.32	67.50	57.58
2018	87.50	83.95	90.29
2019	82.59	85.71	80.40

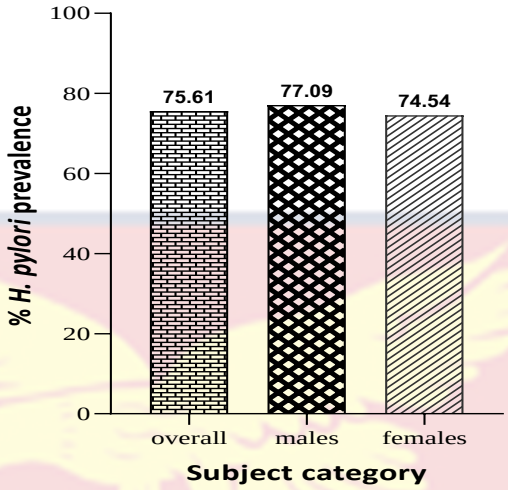


Figure 1: General prevalence of *H. pylori* infection in males and females

Prevalence of *H. Pylori* Infection among People of Different Age Groups

The prevalence of *H. pylori* infection was estimated in participants of different age groups (Figure 2). The rate was estimated to be at least 72 % in all the groups. While an estimate of 72 - 74 % was recorded for the year groups <20 years and those above 61 years, a range of 76 - 78 % in prevalence was realised for ages between 21 years and 60 years (Figure 2).

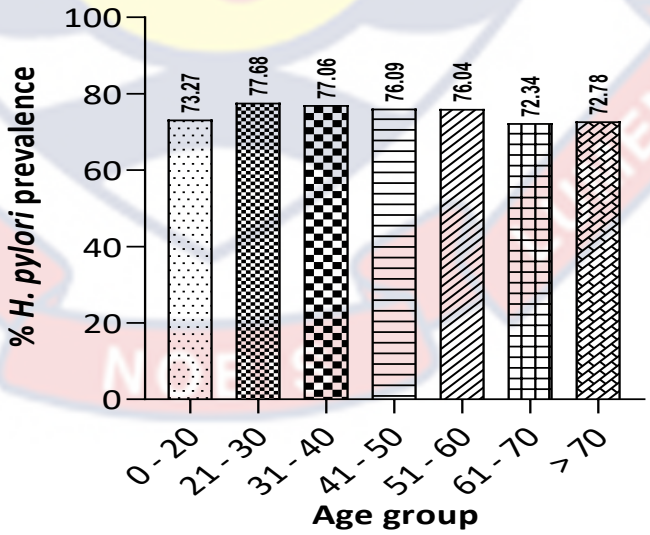


Figure 2: Prevalence of *H. pylori* infection for various age groups

Prevalence of various Gastroduodenal Pathologies in *H. pylori* Infected Males and Females

There are several health conditions that have been linked to *H. pylori* infection. Gastritis was identified as the disease condition with the highest rate of occurrence, with rates of 73.60% and 52.49% in males and females, respectively (Table 3). All reports of esophageal cancer (1.05 %) and retching gastropathy (0.17 %) cases were found to be in men. In addition, males (2.80%) had a far higher prevalence of gastric cancer than did females (0.39%) (Table 3). The second and third most common diseases in both men and women were esophagitis and hiatal hernia, respectively (Table 3).

Table 3: Distribution of Pathological Conditions in *H. pylori* Infected Males and Females

Clinical Condition	Prevalence (%)	
	Males	Females
Gastritis	73.60	52.49
Gastric ulcer	10.66	8.12
Gastric cancer	2.80	0.39
Esophagitis	30.94	30.76
Hiatal hernia	27.45	22.77
Candidiasis	3.15	1.44
Esophageal varices	4.37	1.83
Lax	2.27	3.89
Duodenitis	4.02	3.14
Duodenal ulcer	5.42	3.01
Esophageal cancer	1.05	0.00
P/H gastropathy	0.52	0.13
Retching gastropathy	0.17	0.00
Polyp	0.17	0.13

P/H = portal hypertensive gastropathy

Association between *H. pylori* Infection and Gastroduodenal Pathological Outcomes

The odds of association between endoscopic findings and *H. pylori* infection (table 2) were obtained from analysis of past patient records. Out of the total *H. pylori* positive records (1336/1767), gastritis (822/1336) was the highest pathological diagnosis with a prevalence of 61.53 % (Table 4). Disease conditions such as duodenal and gastric ulcers, gastritis, and esophagitis were all statistically associated with *H. pylori* infection.

Table 4: Odds of association between *H. pylori* infection and pathological outcome

Endoscopic finding	*H. pylori* status		Odds ratio (95 % CI)	*p*-value
	Positive n=1336	Negative n=431		
Gastritis	822 (61.53)	129 (29.93)	3.74 (1.48-4.73)	<0.0001*
Gastric ulcer	123 (9.21)	25 (5.80)	1.65 (1.07-2.57)	0.028*
Gastric cancer	19 (1.42)	2 (0.46)	3.10 (0.81-13.50)	0.130
Esophagitis	412 (30.84)	54 (12.53)	3.11 (2.29-4.25)	<0.0001*
Hiatal hernia	331 (24.78)	79 (18.33)	1.47 (1.12-1.93)	0.006*
Candidiasis	29 (2.17)	5 (1.16)	1.89 (0.77-4.54)	0.228
Lax LES	42 (3.14)	13 (3.02)	1.04 (0.55-1.95)	>0.999
Esoph. varices	39 (2.92)	18 (4.18)	0.69 (0.40-1.23)	0.2108
Duodenitis	47 (3.52)	15 (3.48)	1.01 (0.56-1.84)	>0.9999
Duod. ulcer	54 (4.04)	6 (1.39)	2.98 (1.31-6.47)	0.0058*
Esoph. cancer	6 (0.45)	1 (0.23)	1.94 (0.32-22.33)	>0.9999
Portal H. Gas.	9 (0.67)	8 (1.86)	0.36 (0.15-0.91)	0.0428*
Retching Gas.	1 (0.07)	2 (0.46)	0.16 (0.01-1.39)	0.1493
Gastric Polyp	2 (0.15)	1 (0.23)	0.64 (0.07-9.36)	0.5680
Normal	163 (12.20)	87 (20.19)	0.55 (0.41-0.73)	

Data presented as n (%), where n is frequency. Lax LES = Lax lower esophageal sphincter, Portal H. Gas. = portal hypertensive gastropathy, Duod. ulcer = duodenal ulcer, Recthing Gas. = Retching gastropathy. '*' indicates p-value is statistically significant.

Frequency of Endoscopic Findings in *H. pylori* Patients of different Age Groups

Gastritis was the highest diagnosis across all age groups (Table 5). Group 31 – 40 years recorded the highest gastritis (66.27 %, n = 167/252) while the least was seen in < 20 year group (51.35 %, n = 38/74). Clinical conditions from infection appear to be evenly distributed across the age groups. This implies the infection has somewhat similar effects irrespective of age. Apart from age group <20 years where normal diagnosis was the second most common diagnosis followed by esophagitis and then Hiatal hernia, all other age groups had esophagitis followed by hiatal hernia as the second and third highest respective diagnosis. Both cases of gastric polyp were found among 61-70 years (Table 5). Out of the six (6) esophageal cancer cases, three (2.61 %) were found in age group >70 years. Fifteen (15) out of the nineteen (19) records identified to be diagnosed of gastric cancer occurred in persons more than 51 years with seven (6.09 %) of them found in those greater than 70 years.

Table 5: Frequency of endoscopic findings among *H. pylori* positive patients in the various age groups

Endoscopic Finding	Age Group (Years)						
	0-20 N=74	21-30 N=261	31-40 N=252	41-50 N=226	51-60 N=238	61-70 N=170	>70 N=115
Gastritis	38 (51.35)	158 (60.54)	167 (66.27)	134 (59.29)	145 (60.92)	112 (65.88)	68 (59.13)
Gastric ulcer	2 (2.70)	13 (4.98)	18 (7.14)	21 (9.29)	29 (12.18)	26 (15.29)	16 (13.91)
Gastric cancer	0 (0.00)	0 (0.00)	3 (1.19)	1 (0.44)	5 (2.10)	3 (1.76)	7 (6.09)
Esophagitis	9 (12.16)	66 (25.29)	75 (29.76)	72 (31.86)	78 (32.77)	70 (41.18)	42 (36.52)
Hiatal hernia	8 (10.81)	59 (22.61)	51 (20.24)	54 (23.89)	75 (31.51)	53 (31.18)	31 (26.96)
Esophageal candidiasis	1 (1.35)	4 (1.53)	8 (3.17)	6 (2.65)	3 (1.26)	3 (1.76)	4 (3.48)
Lax lower esoph. Sph.	2 (2.70)	7 (2.68)	11 (4.37)	6 (2.65)	5 (2.10)	6 (3.53)	5 (4.34)
Esophageal varices	2 (2.70)	0 (0.00)	4 (1.59)	14 (6.19)	9 (3.78)	6 (3.53)	3 (2.61)
Duodenitis	2 (2.70)	6 (2.30)	9 (3.57)	11 (4.87)	8 (3.36)	6 (3.53)	5 (4.34)
Duodenal ulcer	2 (2.70)	7 (2.68)	11 (4.37)	3 (1.33)	14 (5.88)	9 (5.29)	11 (9.57)
Esophageal cancer	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.44)	1 (0.42)	1 (0.59)	3 (2.61)
Portal hyp. Gas.	1 (1.35)	0 (0.00)	1 (0.40)	1 (0.44)	1 (0.42)	1 (0.59)	0 (0.00)
Retching gastropathy	0 (0.00)	1 (0.38)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Gastric Polyp	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	2 (1.18)	0 (0.00)
Normal Findings	18 (24.32)	45 (17.24)	26 (10.32)	30 (13.27)	17 (7.14)	16 (9.41)	11 (9.57)

Data presented as n (%), where n is frequency, Lax lower esoph. Sph. = Lax lower esophageal sphincter, portal hyp. Gas. = portal hypertensive gastropathy.

Sociodemographic Characteristics of Study Participants

This study was conducted during the period from October 2019 to August 2021. During the period, 169 patients who were having upper gastrointestinal endoscopies were questioned and provided detailed responses to a number of questions about their personal characteristics and way of life. They were between the ages of 21 and 78. Participants were selected after they satisfied the inclusion criteria. Patients included were from different hospitals across the Central Region of Ghana. About 70% of the population came from Cape Coast and the surrounding area. The remaining individuals came from areas such as Takoradi, Kasoa, and Winneba. The number of females included were more than twice their male counterparts. Majority of the participants were farmers (25.44 %) and traders (28.99 %). A summary of the study participants is provided in the table below (Table 6).

Table 6: Sociodemographic Characteristics of Sampled Patients

	Parameter	Frequency, n (%)
Gender	Male	50 (29.59)
	Female	119 (70.41)
Age group	< 30	27 (15.98)
	30 - 50	55 (32.54)
	51 - 70	68 (40.24)
	> 70	19 (11.24)
Job status	Student	26 (15.38)
	Trading	49 (28.99)
	Farming	43 (25.44)
	Professional	32 (18.93)
	No work	9 (5.33)
Education	No formal education	38 (22.49)
	Primary/Form 4	53 (31.36)
	Secondary	43 (25.44)
	Tertiary	35 (20.71)

Influence of Sociodemographic Factors on *H. pylori* Infection

Patients present for endoscopy procedure at referral centres who consented to partake in the study were provided with questionnaires to fill out. Completed questionnaires were analysed for assessment of sociodemographic factors and their effect on *H. pylori* infection. Records show a significant association between *H. pylori* status and a number of factors (Table 7). Higher levels of relationship are seen in factors such as marital status, duration of indication for endoscopy prior to visit to the facility, and diet restrictions. All these were found to be statistically significant in association with infection. Level of education, intake of vegetable and personal history of dyspepsia were all found to have a significant association with infection (Table 7).

Table 7: Prevalence of *H. pylori* Infection according to Sociodemographic Factors

Variable		*H. pylori* status		*p*-value
		Positive N=103	Negative N=66	
Place of early childhood stay	Village	11 (10.68)	8 (12.12)	0.8061
	Town/city	92 (89.32)	58 (87.88)	
Persons sharing accommodation	<5	40 (38.83)	23 (34.85)	0.6282
	≥5	63 (61.17)	43 (65.15)	
Education level	None	21 (20.39)	17 (25.76)	0.0017**
	Prim/Form 4	40 (38.83)	13 (19.70)	
	Sec	17 (16.50)	26 (39.39)	
	Tertiary	25 (24.27)	10 (15.15)	
Job status	None	8 (7.77)	1 (1.52)	0.0154*
	Student	17 (16.50)	9 (13.64)	
	Trader	25(24.27)	24 (36.36)	
	Farmer	19 (18.45)	24 (36.36)	
	Professional	24 (23.30)	8 (12.12)	
Marital status	Single	22 (21.36)	16 (24.24)	0.0002***
	Married	53 (51.46)	49 (74.24)	
	Divorced	10 (9.71)	1 (1.52)	
	Widowed	18 (17.48)	0 (0.00)	
History of gastrectomy	Yes	0 (0.00)	0 (0.00)	>0.9999
	No	103 (100.0)	66 (100.0)	
Monthly income	None	35 (33.98)	17 (25.76)	0.0712
	<800	37 (35.92)	23 (34.85)	
	800-1600	10 (9.71)	16 (24.24)	
	>1700-3200	21 (20.39)	10 (15.15)	
Currently smoke	Yes	4 (3.88)	0 (0.00)	0.6495
	No	99 (96.12)	66 (100.0)	
Ever smoked	Yes	6 (5.83)	0 (0.00)	0.2486
	No	97 (94.17)	66 (100.0)	
Currently takes alcohol	Yes	9 (8.74)	0 (0.00)	0.0905
	No	94 (91.26)	66 (100.0)	
Previously takes alcohol	Yes	37 (35.92)	20 (30.30)	0.5067
	No	66 (64.08)	46 (69.70)	
Meals per day	1-2times	29 (28.16)	24 (36.36)	0.3087
	>twice	74 (71.84)	42 (63.64)	
Spicy food preference	Yes	42 (40.78)	33 (50.00)	0.2685
	No	61 (59.22)	33 (50.00)	
Diet restriction	Yes	26 (25.24)	36 (54.55)	0.0002***

Table 7, continued

	No	77 (74.76)	30 (45.45)	
On antimicrobials	Yes	24 (23.30)	26 (39.39)	0.0376*
	No	79 (76.70)	40 (60.61)	
Pre-soften food for babies	Yes	5 (4.85)	0 (0.00)	0.4058
	No	98 (95.15)	66 (100.0)	
Vegetable/fruit intake	Daily	24 (23.30)	7 (10.61)	0.0426*
	Sometimes	79 (76.70)	59 (89.39)	
History of surgery for gastrectomy	Yes	0 (0.00)	0 (0.00)	>0.9999
	No	103 (100.0)	66 (100.0)	
Regular exercise	Yes	42 (40.78)	26 (39.39)	0.8738
	No	61 (59.22)	40 (60.61)	
Source of drinking water	Tap	19 (18.45)	18 (27.27)	0.0142*
	Sachet	58 (56.31)	43 (65.15)	
	Sachet/tap	17 (16.50)	1 (1.52)	
	River/borehole / well	8 (7.77)	4 (6.06)	
Duration of symptom	Few weeks	15 (14.56)	36 (54.55)	<0.0001****
	< 6months	21 (20.39)	15 (22.73)	
	At least a year	66 (64.08)	15 (22.73)	
Family dyspepsia history	Yes	10 (9.71)	4 (6.06)	0.0393*
	No	46 (44.66)	19 (28.79)	
	Don't know	46 (44.66)	43 (65.15)	
Personal dyspepsia history	Yes	29 (28.16)	29 (43.94)	0.0461*
	No	74 (71.84)	37 (56.06)	
Knowledge on infection	Yes	10 (9.71)	2 (3.03)	0.1296
	No	93 (90.29)	64 (96.97)	
How infection is transmitted	Yes	9 (8.74)	0 (0.00)	0.0905
	No	94 (91.26)	66 (100.0)	

ns = not significant, sec = secondary, prim. = primary. '*' indicates level of significance where '*' is the least and '****' is the highest.

Electrophoregrams of Detected Virulence Genes

Extracted DNA from gastric biopsies were amplified using specific primers and electrophoresed on 2 % agarose gel. Electrophoregrams were obtained after gels were viewed under UV transilluminator. The presence of *H. pylori* was reported when amplified gene corresponded to 768bp (Figure 3, A-E). Virulence genes were also analysed and electrophoregrams recorded (Figure 3, A-E). *dupA* and *cagE* were observed at amplicon sizes of 662bp and 500bp respectively. *iceA2* was observed at either 334bp or 228bp (Figure 3, A-E). From Figure 4 (A-C), the detection of *vacAi1*, *vacAm1*, and *vacAm2* are illustrated on 1.5% agarose gel.

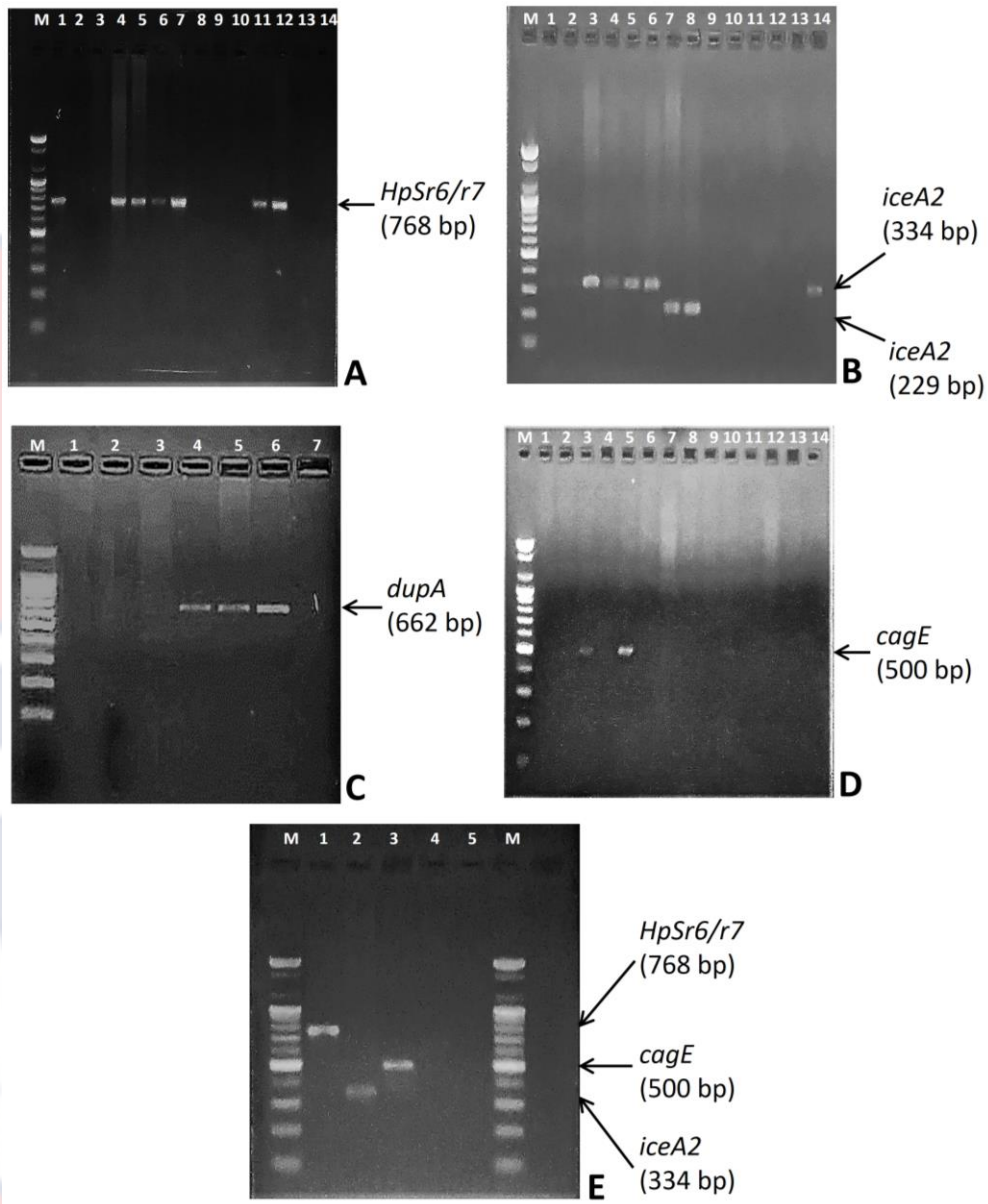


Figure 3: Electrophoregrams for *H. pylori* detection, and *iceA*, *dupA* and ***cagE*** virulence genes

Ethidium bromide-stained 1.5 % agarose gel electrophoregram showing various PCR products and molecular weight markers (Lane M, 100 bp molecular ladder). (**A**: Confirmation of *H. pylori* DNA samples using *HpSr6/r7* primers. Lane 1, 4-7, 11, 12 - presence of *H. pylori*; lanes 2, 3, 8-10, 13 – absence of *H. pylori*. lane 14 - Molecular grade water (negative control). **B**: Detection of *iceA2* genes. lanes 3 – 8, 14 - presence of *iceA2* genes; lane 1,2, 9-13 - absence of *iceA2* gene. **C**: detection of *dupA* gene. lane 4-6 presence of *dupA* gene; lane 1-3, and 7 absence of *dupA* gene. **D**: detection of *cagE* gene. lanes 3, 5 - presence of *cagE* gene; lanes 1, 2, 4, 6-14 - absence of *cagE* gene; **E**: detection of *dupA*, *cagE*, and *HpSr6/r7*. Lane 1 - *HpSr6/*r7; lane 2 – *iceA2* gene; lane 3 – *cagE* gene; lane 4, 5 – negative control samples.

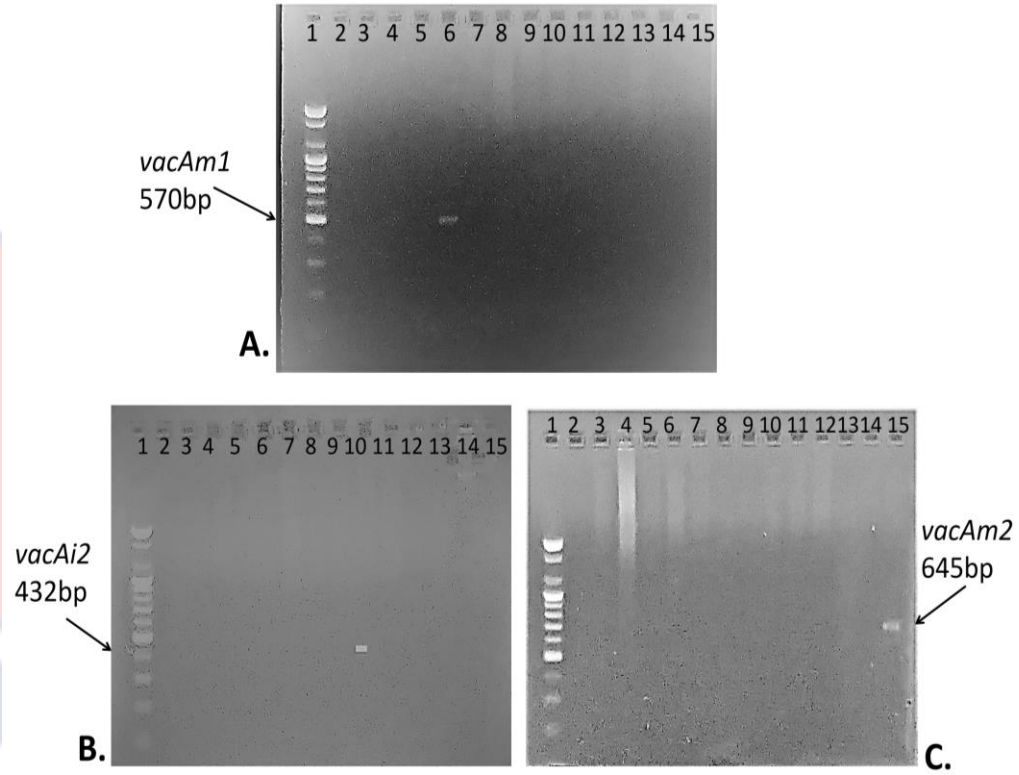


Figure 4: Electrophoregrams for *vacAi2*, *vacAm1* and *vacAm2* virulence genes

Ethidium bromide-stained 1.5 % agarose gel electrophoregram showing various PCR products and molecular weight markers (Lane 1 displays various bands obtained for 100 bp molecular ladder). **A**; Lane 6 indicates the detection of *vacAm1* corresponding to 570bp on the ladder, **B**; Lane 10 shows a band at 432bp indicating the detection of *vacAi2*. **C**; Lane 15 shows a band at 645bp indicating the detection of *vacAm2*. All other lanes are showing negative results.

Prevalence of *H. pylori* Virulence Factors in Males and Females

Virulence factors investigated included *cagE, dupA, iceA,* and some sub-types of *vacA* gene such as *m1, m2, i2.* The distribution of the genes in males and females (Table 8), frequency of occurrence of gene combinations in patients (Table 9) and the odds of association between the genes and related clinical conditions (Tables 10 to 15) were investigated. The most prevalent virulent factors were *iceA2* (28.16 %) and *dupA* (32.04 %) (Table 8). Apart from *dupA*

and *vacAi2* which recorded higher rates in females than males, all other virulence factors recorded a higher detection rate in males.

The appearance of different subtypes for the same region (eg. both *s1* and *s2* or *m1* and *m2* detected in a single sample) of *vacA* gene is an indication of mixed infection. Results on genotype combinations in infected individual shows mixed infection in some patients. The concurrent appearance of *m1* and *m2* in participants (as shown by the genotype combinations *dupA+vacAm1+vacAm2*, *m1+m2*, and *cagE+iceA2+vacAm1+vacAm2*) indicates a mixed infection (Table 9). *IceA2* (14.56 %) and *dupA* (18.45 %) were the most presented in terms of lone virulent factors per participant (Table 9).

Table 8: Distribution of virulence factors and prevalence in males and females

H. pylori Genotypes	Total, n % N=103	Males, n % N=40	Females, n % N=63
cagE	13 (12.62)	7 (17.50)	6 (9.52)
dupA	33 (32.04)	10 (25.00)	23 (36.51)
iceA2	29 (28.16)	17 (42.50)	12 (19.05)
vacAi2	3 (2.91)	1 (2.50)	2 (3.17)
vacAm1	8 (7.77)	6 (15.00)	2 (3.17)
vacAm2	3 (3.88)	2 (5.00)	1 (1.59)

Table 9: Frequency of occurrence of genotype combinations in respondents

H. pylori genotypes	Number	% Prevalence
cagE only	5	4.85
cagE+dupA only	3	2.91
cagE+iceA2 only	2	1.94
cagE+iceA2+dupA	1	0.97
iceA2 only	15	14.56
iceA2+dupA only	5	3.88
iceA2+dupA+vacAi2 only	2	2.91
dupA only	19	18.45
dupA+vacAm1+vacAm2 only	2	1.94
cagE+dupA+iceA2+i2 only	1	0.97
vacAm1+vacAm2 only	1	0.97
m1 only	2	1.94
iceA2+m1 only	3	2.91
cagE+iceA2+vacAm1+vacAm2 only	1	0.97

Odds of Association between Virulence Genes and Clinical Outcomes

Odds of association between virulent factors and clinical outcome of infection are shown on tables 10 to 15. *IceA2* recorded a significant association with duodenal ulcer and gastritis (Table 11). No clinical condition was significantly associated with *cagE* (Table 12) and *dupA* (table 10). *dupA* however, showed higher odds of association with varices (6.90; CI: 0.975 - 90.60) (Table 10). *dupA* had no significant association with duodenal ulcer, recording odds of association to be 2.43 (CI: 0.374 to 30.40).

A significant association was obtained for the presence of *vacAm1* and gastric and duodenal ulcers as well as varices (Table 13). Varices showed a significant association with *vacA m2*. There was no other clinical condition observed to be significantly associated with *m2*. Odds of association between gastric cancer

and *m1* was 6.64 (CI: 0.405 to 60.40). *m1* showed no other relationship with clinical conditions. No significant association between *vacAi2* and clinical condition of *H. pylori* infection was found (Table 15).

Prevalence of clinical conditions in relation to antibiotic resistance genes are presented in table 16. Primer sets that has been reported in earlier studies (Table 1) for the detection of *H. pylori* antibiotic resistant genes were used to amplify regions within *rdxA*, *gyrA*, and *pbp* genes. These genes, according to research, play a role in the development of antibiotic resistance to metronidazole, levofloxacin, and amoxicillin respectively.

Table 10: Odds of association between *dupA* gene and pathological outcome

Endoscopic finding	*dupA* status		OR (CI)	*p value*
	+ve	**-ve**		
Gastritis	20	45	0.855 (0.370 to 2.00)	0.8273
G. ulcer	7	7	2.42 (0.821 to 7.10)	0.1346
Duodenitis	0	3	0.00 (0.00 to 2.44)	0.5494
D. ulcer	4	1	2.43 (0.374 to 30.40)	0.6492
HH	5	13	0.783 (0.286 to 2.273)	0.7854
Varices	3	1	6.90 (0.975 to 90.60)	0.0956
Esophagitis	11	12	2.42 (0.900 to 6.38)	0.0791
G. cancer	1	2	1.06 (0.0713 to 9.39)	>0.9999
Lax LES	0	1	0.00 (0.00 to 19.10)	>0.9999
Normal	2	14	0.258 (0.056 to 1.12)	0.0843

G. ulcer = gastric ulcer, HH = hiatal hernia, D. ulcer = duodenal ulcer. G. cáncer = gastric cáncer, Lax LES = Lax Lower Esophageal Sphincter

Table 11: Odds of association between *iceA2* gene and pathological outcome

Endoscopic Finding	*iceA2* status		OR (CI)	*p value*
	+ve	**-ve**		
Gastritis	26	39	7.78 (2.27 to 25.70)	0.0005***
G. ulcer	6	8	2.15 (0.632 to 6.25)	0.2099
Duodenitis	0	3	0.00 (0.00 to 2.94)	0.5571
D. ulcer	4	1	11.7 (1.74 to 144)	0.0214*
HH	5	13	0.978 (0.354 to 2.78)	>0.9999
Varices	0	4	0.00 (0.00 to 2.64)	0.5748
Esophagitis	10	13	2.47 (0.972 to 6.64)	0.0721
G. cancer	1	2	1.29 (0.086 to 11.40)	>0.9999
Lax LES	0	1	0.00 (0.00 to 23.00)	>0.9999
Normal	1	15	0.14 (0.013 to 0.821)	0.0365*

G. ulcer = gastric ulcer, HH = hiatal hernia, D. ulcer = duodenal ulcer. G. cáncer = gastric cancer, Lax LES = Lax Lower Esophageal Sphincter

Table 12: Odds of association and relative risk of *cagE* gene and pathological outcomes

Endoscopic Finding	*cagE* status		OR	*p value*
	+ve	**-ve**		
Gastritis	6	59	0.450 (0.132 to 1.38)	0.2222
G. ulcer	3	11	2.15 (0.562 to 8.51)	0.3791
Duodenitis	1	2	3.67 (0.235 to 32.80)	0.3357
D. ulcer	1	4	1.79 (0.136 to 12.70)	0.498
HH	1	17	0.36 (0.0317 to 2.35)	0.4565
Varices	0	4	0.00 (0.00 to 7.73)	>0.9999
Esophagitis	2	21	0.597 (0.125 to 2.47)	0.7276
G. cancer	0	3	0.00 (0.00 to 8.20)	>0.9999
Lax LES	0	1	0.00 (0.00 to 62.30)	>0.9999
Normal	0	16	0.00 (0.00 to 1.32)	0.2116

G. ulcer = gastric ulcer, HH = hiatal hernia, D. ulcer = duodenal ulcer. G. cáncer = gastric cancer, Lax LES = Lax Lower Esophageal Sphincter

Table 13: Odds of association between *vacAm1* and pathological outcome

Endoscopic Finding	*vacA m1* status		OR	*p value*
	+ve	-ve		
Gastritis	4	61	0.557 (0.155 to 2.03)	0.4627
G. ulcer	5	9	15.9 (3.15 to 64.50)	0.001**
Duodenitis	0	3	0.00 (0.00 to 14.50)	>0.9999
D. ulcer	3	2	27.9 (4.44 to 168)	0.0029**
HH	0	18	0.00 (0.00 to 1.79)	0.1968
Varices	2	1	31.3 (3.01 to 454)	0.0154*
Esophagitis	1	22	0.474 (0.04 to 2.96)	0.6802
G cancer	1	2	6.64 (0.405 to 60.40)	0.2173
Lax LES	0	1	0.00 (0.00 to 107)	>0.9999
Normal	0	16	0.00 (0.00 to 2.54)	0.3484

G. ulcer = gastric ulcer, HH = hiatal hernia, D. ulcer = duodenal ulcer. G. cáncer = gastric cancer, Lax LES = Lax Lower Esophageal Sphincter

Table 14: Odds of association between *vacAm2* and pathological outcomes

Endoscopic Finding	*vacA m2* status		OR	*p value*
	+ve	**-ve**		
Gastritis	3	62	1.79 (0.258 to 23.80)	>0.9999
G. ulcer	0	14	0.00 (0.00 to 7.03)	>0.9999
Duodenitis	0	3	0.00 (0.00 to 34.00)	>0.9999
D. ulcer	0	5	0.00 (0.00 to 29.20)	>0.9999
HH	0	18	0.00 (0.00 to 5.07)	>0.9999
Varices	2	1	98.0 (7.07 to 1403)	0.0034*
Esophagitis	0	23	0.00 (0.00 to 3.66)	0.5726
G cancer	0	3	0.00 (0.00 to 34.00)	>0.9999
Lax LES	0	1	0.00 (0.00 to 223)	>0.9999
Normal	0	16	0.00 (0.00 to 5.91)	>0.9999

G. ulcer = gastric ulcer, HH = hiatal hernia, D. ulcer = duodenal ulcer. G. cáncer = gastric cancer, Lax LES = Lax Lower Esophageal Sphincter

Table 15: Odds of association and relative risk of *vacAi2* with pathological outcomes

Endoscopic Finding	*vacA i2* status		OR	*p value*
	+ve	**-ve**		
Gastritis	3	62	1.79 (0.258 to 23.80)	>0.9999
G. ulcer	1	13	2.21 (0.159 to 15.60)	0.4478
Duodenitis	0	3	0.00 (0.00 to 21.10)	>0.9999
D. ulcer	1	4	7.92 (0.495 to 62.20)	0.183
HH	2	16	5.19 (0.752 to 34.20)	0.1399
Varices	0	3	0.00 (0.00 to 34.00)	>0.9999
Esophagitis	0	23	0.00 (0.00 to 3.66)	0.5726
G cancer	0	3	0.00 (0.00 to 34.00)	>0.9999
Lax LES	0	1	0.00 (0.00 to 223)	>0.9999
Normal	0	16	0.00 (0.00 to 5.91)	>0.9999

G. ulcer = gastric ulcer, HH = hiatal hernia, D. ulcer = duodenal ulcer.

Percentage Occurrence of Antibiotic Resistance Genes

GyrA for levofloxacin resistance was the most represented resistant gene (67.96 %) followed by *rdxA* (45.63 %) for metronidazole and *pbp* (35.92 %) (Table 16). While majority of the detected *gyrA* gene (42.86 %) were found to be present alone in infected individuals, the *rdxA* and *pbp* genes were found to mostly appear together with another gene. Only 21.28 % of the detected *rdxA* gene and 18.92 % of those of *pbp1* were found to exist as a single gene per infected individual. The highest combination was realised for the occurrence of all three genotypes (16.50 %) (Table 16).

Table 16: Percentage Occurrence of Antibiotic Resistance Gene Combinations

Gene Combination	Number	% Occurrence
Overall *rdxA* detected	47	45.63
Overall *gyrA* detected	70	67.96
Overall *pbp1* detected	37	35.92
rdxA+*pbp1* only	5	4.85
rdxA+*gyrA* only	15	14.56
pbp1+*gyrA* only	8	7.77
rdxA+*pbp1*+*gyrA*	17	16.50
rdxA only	10	9.71
pbp1 only	7	6.80
gyrA only	30	29.13
None	58	56.31

Electrophoregrams for Antibiotic Resistance Genes

Extracted DNA from gastric biopsies were amplified using specific primers and electrophoresed on 2 % agarose gel. Electrophoregrams were obtained after gels were viewed under UV transilluminator. The presence of various antibiotic resistance genes were confirmed to be present when the amplified genes *rdxA*, *gyrA*, and *pbp1A* were observed to correspond to amplicon sizes 880bp, 582bp and 612bp respectively (Figure 5). All bands were compared to 100bp reference molecular weight ladder (Bioneer, USA).

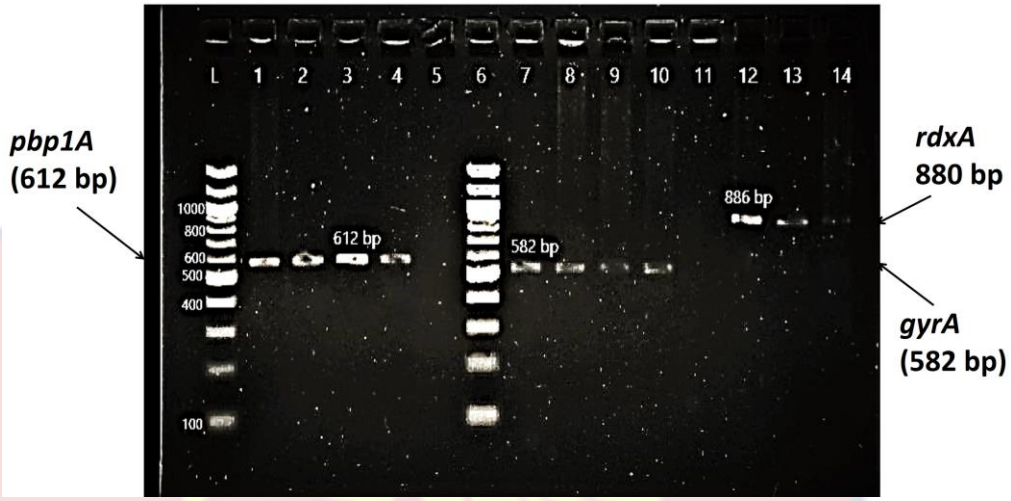


Figure 5: Electrophoregrams for *pbp1A*, *rdxA* and *gyrA* antibiotic resistance genes
Ethidium bromide-stained 1.5 % agarose gel electrophoregram showing various PCR products and molecular weight markers (Lane L and 6, 100 bp molecular ladder). Detection of *pbp1A* genes. lanes 1-4. Detection of *gyrA* gene: lane 7-10. Detection of *rdxA* gene: lanes 12-14.

Antibiotic Resistance Genes Distribution in various Disease Conditions

All GERD conditions had *pbp* and *rdxA* resistant genes and all duodenal ulcer cases had *rdxA* gene (Table 17). 33.3 % of patients with duodenitis had a combination of all three genes. Another condition with all three genes was hiatal hernia (HH) (Table 17).

Complete resistance (100 %) to antibiotics was recorded for metronidazole, amoxicillin, clarithromycin, and amoxicillin-clavulanic acid (Table 18). Among the 15 samples tested and analysed against the various antibiotics, total resistance to all antibiotics was observed for 3 samples. Susceptibility was recorded at 80% for each of tetracycline and ciprofloxacin (Table 18).

Table 17: Distribution of Antibiotic Gene Occurrence in various Gastro-Duodenal Pathologies

Gene Combination	% Occurrence of Antibiotic Resistance Genes in various Disease Conditions							
	Gast. n=65	GU n=14	DU N=5	DN N=3	GC N=3	HH N=18	GERD N=23	Var N=3
rdxA	64.3	40.0	100.0	66.7	50.0	13.0	100.0	64.3
gyrA	35.7	40.0	0.0	0.0	11.1	60.9	66.7	35.7
pbp1	0.0	0.0	0.0	0.0	0.0	21.7	100.0	0.0
rdxA+pbp1	0.0	0.0	33.3	66.7	16.7	0.0	66.7	0.0
rdxA+gyrA	14.3	0.0	0.0	0.0	11.1	4.3	33.3	14.3
pbp1+gyrA	21.4	40.0	0.0	0.0	0.0	8.7	33.3	21.4
rdxA+pbp1+gyrA	0.0	0.0	0.0	33.3	0.0	8.7	0.0	0.0
rdxA only	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
pbp1 only	28.6	0.0	66.7	0.0	22.2	4.3	0.0	28.6
gyrA only	35.7	0.0	0.0	0.0	50.0	39.1	0.0	35.7

Gast. = gastritis, GU = gastric ulcer, DU = duodenal ulcer, DN = duodenitis, GC = gastric cancer, HH = hiatal hernia, EC = esophageal candidiasis, var = varices.

Table 18: Percentage Resistance and Susceptibility Rates obtained for various Antibiotics

Antibiotic tested	Susceptible		Resistant	
	N	**%**	***N***	**%**
Metronidazole	0	0	15	100
Tetracycline	12	80	3	20
Amoxicillin	0	0	15	100
Ciprofloxacin	12	80	3	20
Clarithromycin	0	0	15	100
Amoxicillin-Clavulanic acid	0	0	15	100
Levofloxacin	9	60	6	40

LEV = levofloxacin, TET = tetracycline, CIP = ciprofloxacin, MTZ = metronidazole, CLR = clarithromycin, AMC = amoksiclav, AMX = amoxicillin.

Sequencing results showing Nucleotide Changes and Amino Acid Substitutions as a result of Mutations

PCR products of amplified antibiotic genes were sequenced and analysed for mutations. The presence of amino acid substitutions resulting for mutations were determined and annotated. Various non-synonymous mutations were identified in *gyrA*, *pbp*, and *rdxA* genes (Table 19). Annotations of substituted amino acids on protein domain, alignment with reference strains, and prediction of some secondary and tertiary structures are presented (Figure 6 - 16). A number of mutations were identified in all three genes with the highest occurring in *rdxA* (Table 19). Fewer substitutions were identified in *gyrA* which was generally of high susceptibility against isolates in the sensitivity test conducted (found in table 18). All isolates recorded amino acid substitution in *rdxA* at positions A183V, R90G, and G98S. Multiple allelic mutation was identified for *gyrA* at position 199. In all identified mutations, the highest nucleotide substitution was observed for C to T.

Table 19: Representation of Mutations Indicating Type and Position of Nucleotide Change and Resultant Amino Acid Change

Gene	Nucleotide Position	Nucleotide Change	N (%)	Amino Acid Change
GyrA (ASM878v1)	715369	C/T	1(10.0)	p.(H200Y)
	715367	G/A	1(10.0)	p.(A199=)
	715366	C/T	1(10.0)	p.(A199V)
	715365	G/A	1(10.0)	p.(A199T)
	715340	G/T	1(10.0)	p.(R190S)
	715335	C/T	1(10.0)	p.(H189Y)
	714760	C/T	1(10.0)	p.(A97V)
PBP1 (ASM878v1)	592741	C/T	1(10.0)	p.(T254I)
	592559	A/G	1(10.0)	p.(K315E)
	592186	C/T	2(20.0)	p.(T438M)
RdxA (ASM852v1)	1014155	C/T	1(14.3)	p.(Q11Stop)
	1014139	G/A	1(14.3)	p.(R16H)
	1014106	A/G	1(14.3)	p.(E27G)
	1014079	T/C	1(14.3)	p.(I36T)
	1014038	C/T	2(28.6)	p.(Q50Stop)
	1014004	A/G	1(14.3)	p.(D61G)
	1013986	C/G	1(14.3)	p.(A67G)
	1013978	A/C	1(14.3)	p.(S70R)
	1013974	A/T	2(28.6)	p.(Y71F)
	1013963	G/A	2(28.6)	p.(E75K)
	1013932	T/G	2(28.6)	p.(V85G)
	1013917	G/A	7(100.0)	p.(R90G)
	1013894	G/A	7(100.0)	p.(G98S)
	1013890	A/C	2(28.6)	p.(H99P)
	1013638	C/T	7(100.0)	p.(A183V)
	1013597	C/A	6(85.7)	p.(Q197K)

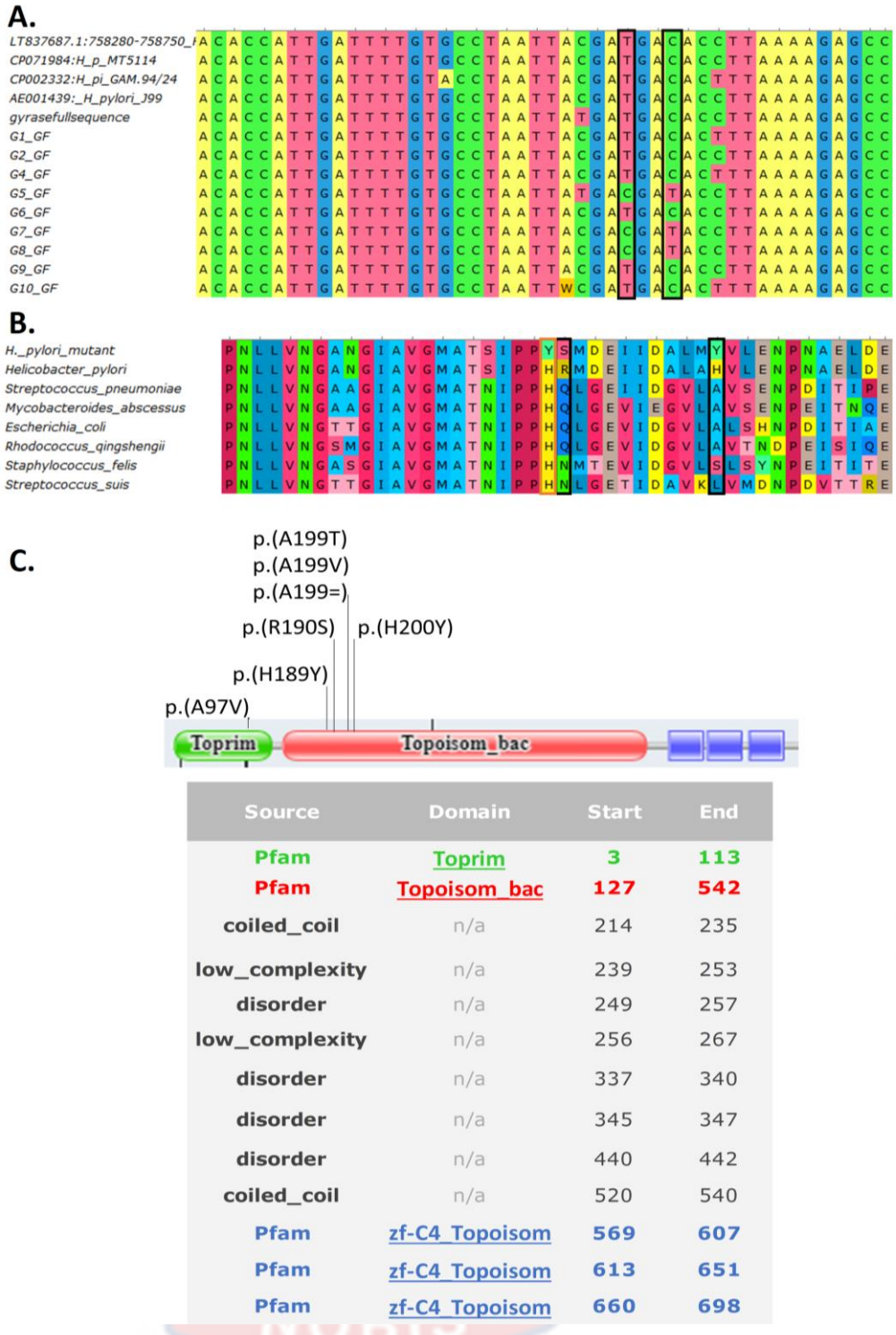
Alignment, Annotation of Mutations on Protein Domain and Prediction of both Secondary and Tertiary Structures of Wild Type and Mutant g*yrA* Gene

Identified mutations were further analysed by predicting the structural changes that will result from those substitutions (Figure 6 – 16). Such structural modifications may have effect on the binding abilities of antibiotics onto bacterial cell walls to effectively perform a function. Alignment of sequenced genes with references shows the presence of variants at various positions (shown with black rectangles in A) (Figure 6). Mutations are observed within the rectangles where substitutions of T for C and C for T are seen for three samples (A). B shows aligned *gyrA* gene from different organisms including *H. pylori* wild type showing conserved regions (indicated with purple rectangle in B where histidine was substituted for tyrosine) and variable regions (indicated with red rectangle) where a similar substitution of histidine for tyrosine was found. All variants are clearly annotated in Figure 6-C showing the domain of *H. pylori* gyrase protein.

Secondary structures generated for wild type (shown in A) and A97V mutant (shown in B) reveals regions of modifications (indicated with rectangles) as a result of the variant (Figure 7). Differences are in seen in the appearance of a helix (indicated with black rectangle) and the deletion of same (indicated with red rectangle) in the mutant as compared to the wild type. Tertiary structures presented in the format of a cartoon (left) and surface (right) of the wild type (shown in C) and mutant (shown in D) when aligned or merged (E) reveals

conformational changes at certain areas of the protein (indicated with red arrows). These changes were due to the substitution of Alanine for Valine at position 97 (Figure 7).

Secondary structures generated for wild type (A) and H189Y mutant (B) showing a single region of change where a helix in the wild type is seen to have shortened in the mutant as a result of the mutation (indicated with black rectangle in A and B) (Figure 8). Tertiary structures presented in cartoon (left) and surface (right) formats of wild type (C) and mutant (D) when aligned or merged (E) reveals conformational changes at certain areas of the protein (indicated with red arrows) due to the substitution of histidine to tyrosine (Figure 8).



Source	Domain	Start	End
Pfam	Toprim	3	113
Pfam	Topoisom_bac	127	542
coiled_coil	n/a	214	235
low_complexity	n/a	239	253
disorder	n/a	249	257
low_complexity	n/a	256	267
disorder	n/a	337	340
disorder	n/a	345	347
disorder	n/a	440	442
coiled_coil	n/a	520	540
Pfam	zf-C4_Topoisom	569	607
Pfam	zf-C4_Topoisom	613	651
Pfam	zf-C4_Topoisom	660	698

Figure 6: Sequenced gyrA-reference strain alignment and annotation of mutations on protein domain

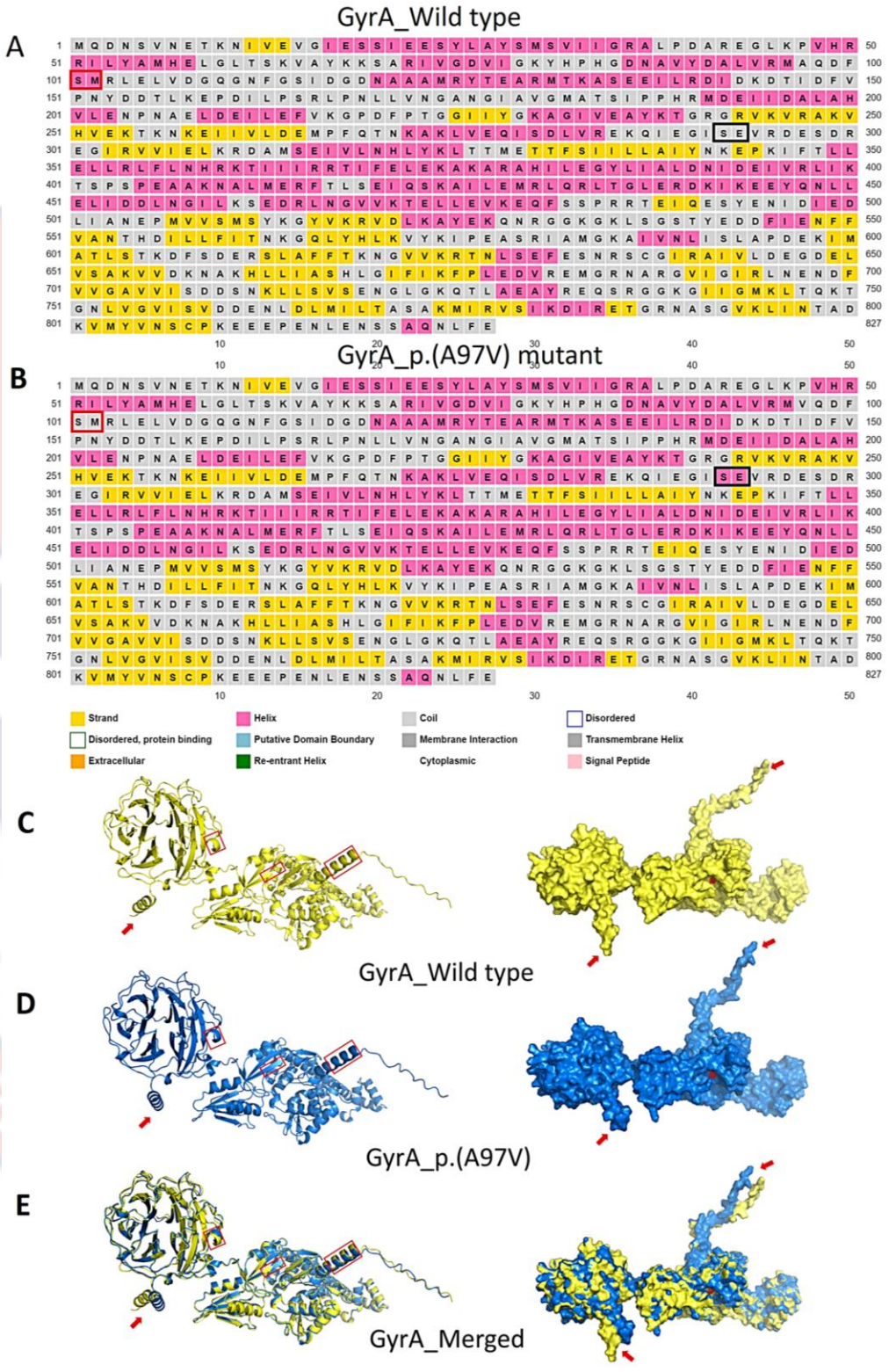


Figure 7: Predicted secondary and tertiary structures of wild type and A97V mutant

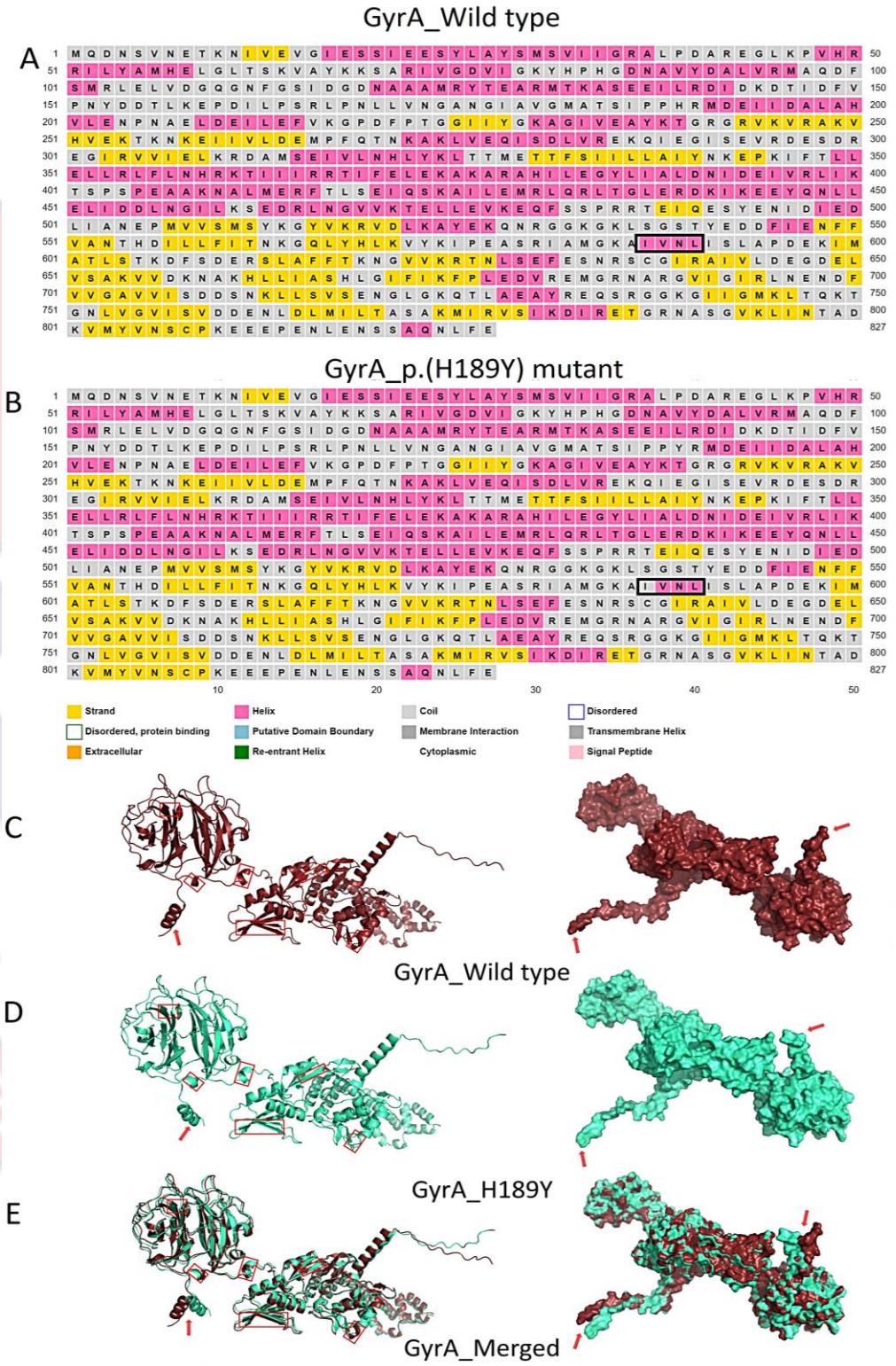


Figure 8: Predicted secondary and tertiary structures of wild type and H189Y mutant

Alignment, Annotation of Mutations on Protein Domain and Prediction of both Secondary and Tertiary Structures of Wild Type and Mutant *pbp1A* Gene

Alignment of sequenced *pbp* gene from isolated *H. pylori* samples against reference genomes reveals variants at various positions (shown with black rectangles in A) (Figure 9A). All variants were identified beyond the transglycosylase regions with a few within the transpeptidase domain (Figure 9B) where catalysis of peptide cross-linking takes place. Variants were annotated in the domain of the protein in B. Overall, there were three (3) non-synonymous mutations namely T254I, K315E, T438M (Figure 9B).

Secondary structures generated for wild type (A) and T254I mutant (B) showing two areas where helices appear to shorten (shown with black rectangle) or elongates (shown with brown rectangle) in the mutant when compared to the wild type (Figure 10 A and B). Tertiary structures presented in cartoon (left) and surface (right) formats of wild type (C) and mutant (D) when aligned or merged (E) reveals conformational changes at certain areas of the protein (indicated with red squares) due to the substitution of threonine for isoleucine (Figure 10).

Three (3) regions of modification are observed when the secondary structures of the wild type (A) and K315E mutant (B) are compared (Figure 11). There is either a shortened strand (indicated with red rectangle) or totally deleted one (indicated with green rectangle) in mutant compared to the wild type (Figure 11). A helix elongation is observed in the mutant (shown with black rectangle).

Tertiary structures presented in cartoon (shown on the left) and surface (shown on the right) formats of wild type (C) and mutant (D) when aligned or merged (E) reveals conformational changes at certain areas of the protein (Figure 11). Modified areas are compared and shown with rectangles.

Secondary structures generated for wild type (A) and T438M mutant (B) reveals different areas in mutant where strands are either shortened (shown with green rectangle), elongated (brown rectangle) or totally deleted (blue rectangle) when compared to the wild type (Figure 12). The amino acid substitution also resulted in the shortening of helices (shown with red and purple rectangles) in some areas and elongation (shown with black rectangle) at another region. In the tertiary structures (C – E) where the cartoon (left) and surface (right) structures are presented, clear changes in structure are seen in the surface structure format (shown with black rectangles) when there was a substitution of threonine for methionine in the mutant. The cartoon structures also show missing (indicated with black and green squares) helices in the mutant when compared to wild type. The yellow rectangle shows an area of shorter length for the mutant compared to wild type.

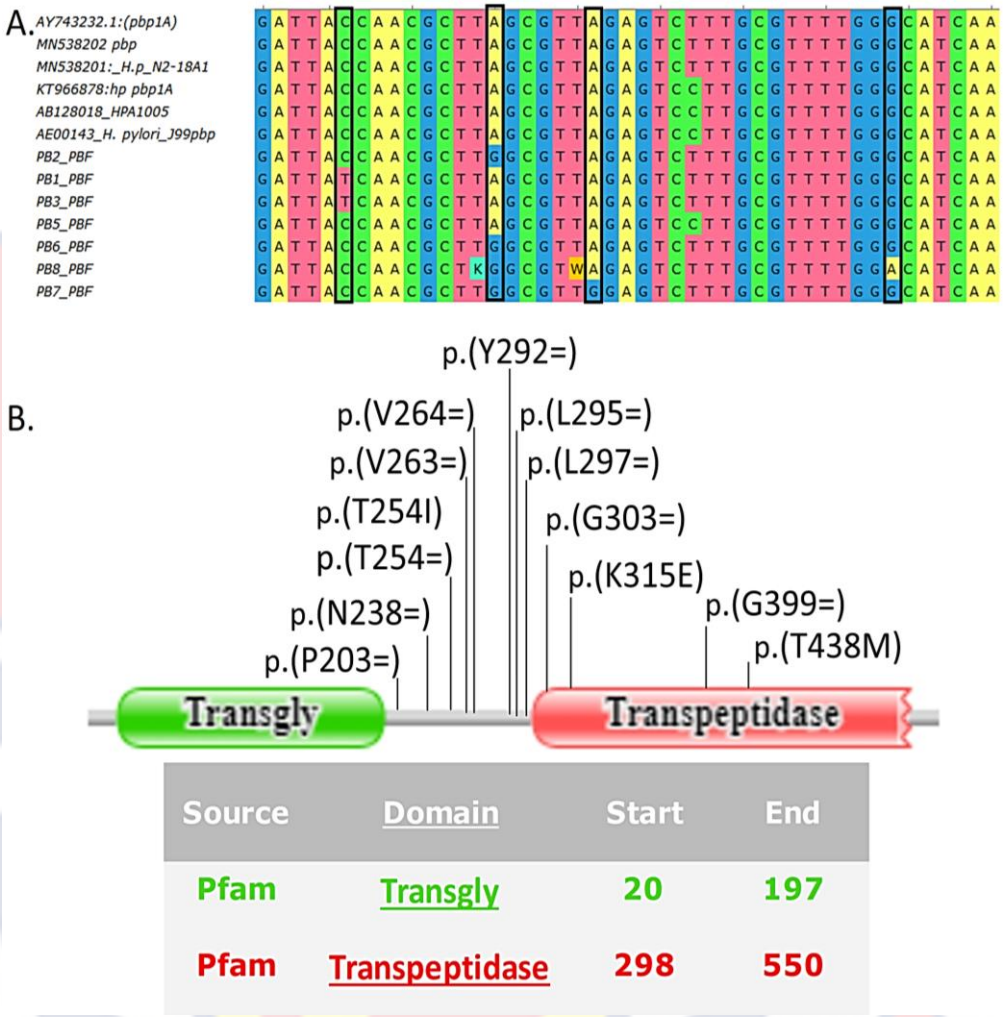


Figure 9: Sequenced pbp -reference strain alignment and annotation of mutations on protein domain

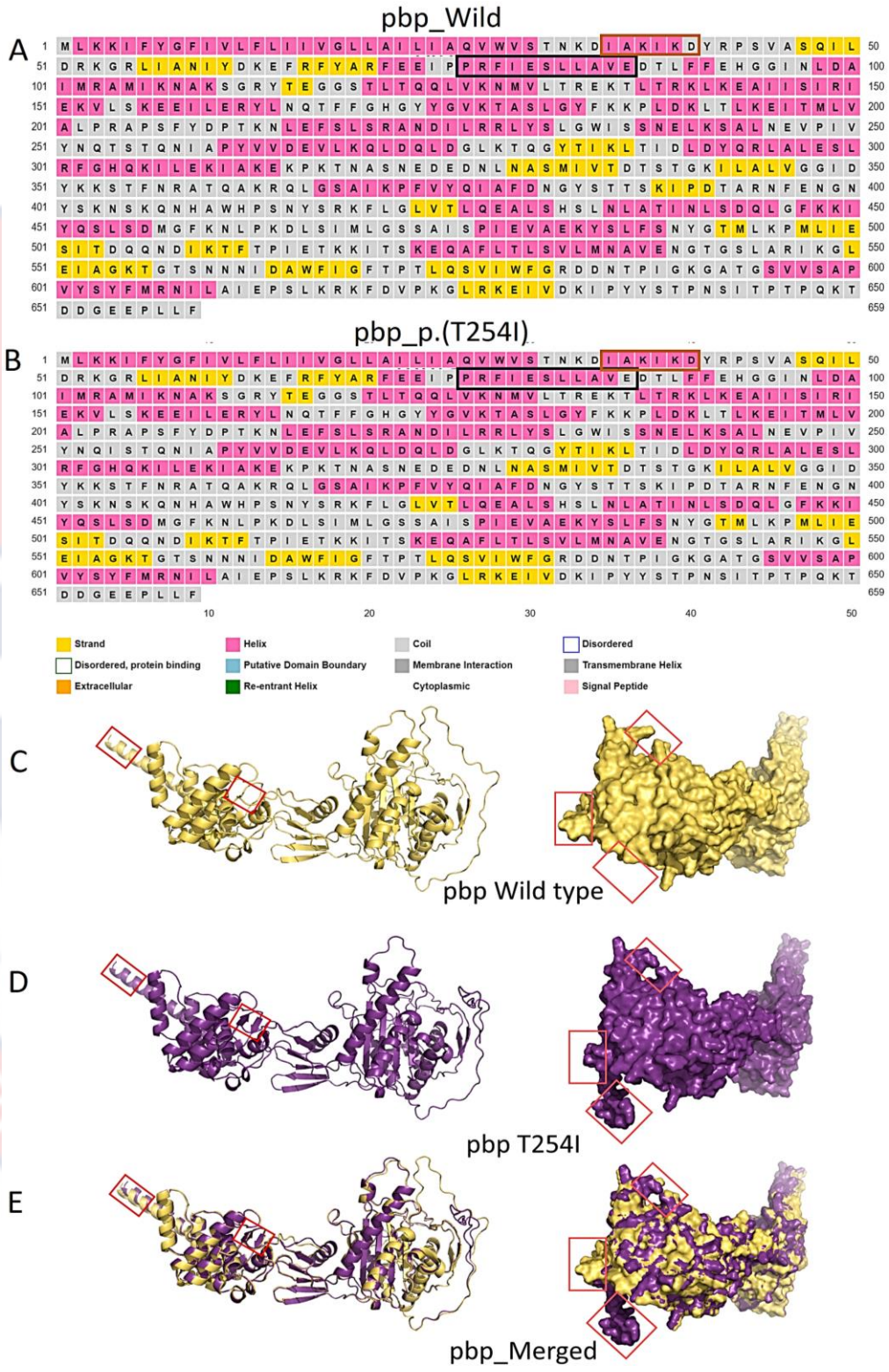


Figure 10: Predicted secondary and tertiary structures of wild type and T254I mutant

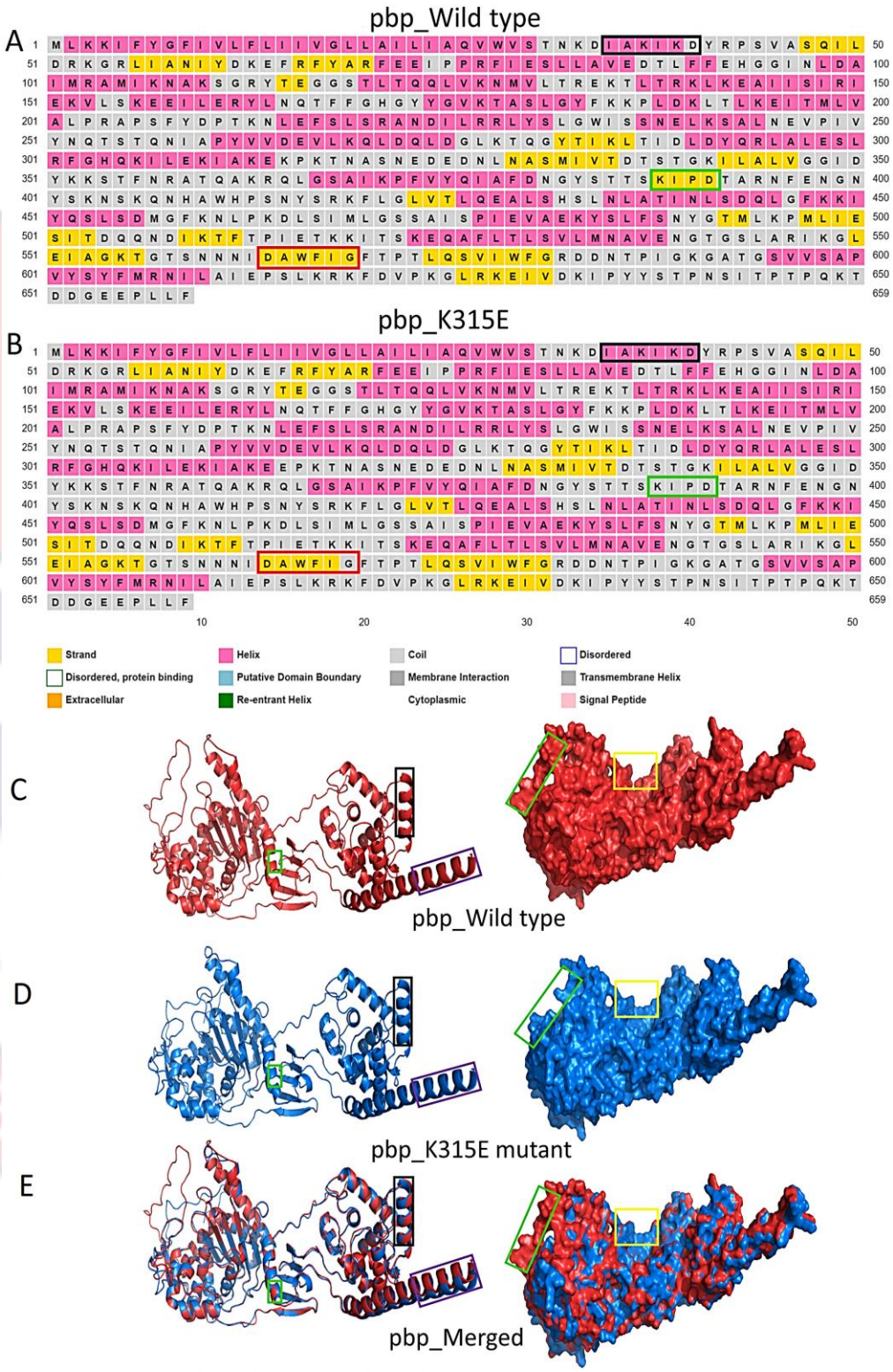


Figure 11: Predicted secondary and tertiary structures of wild type and K315E mutant

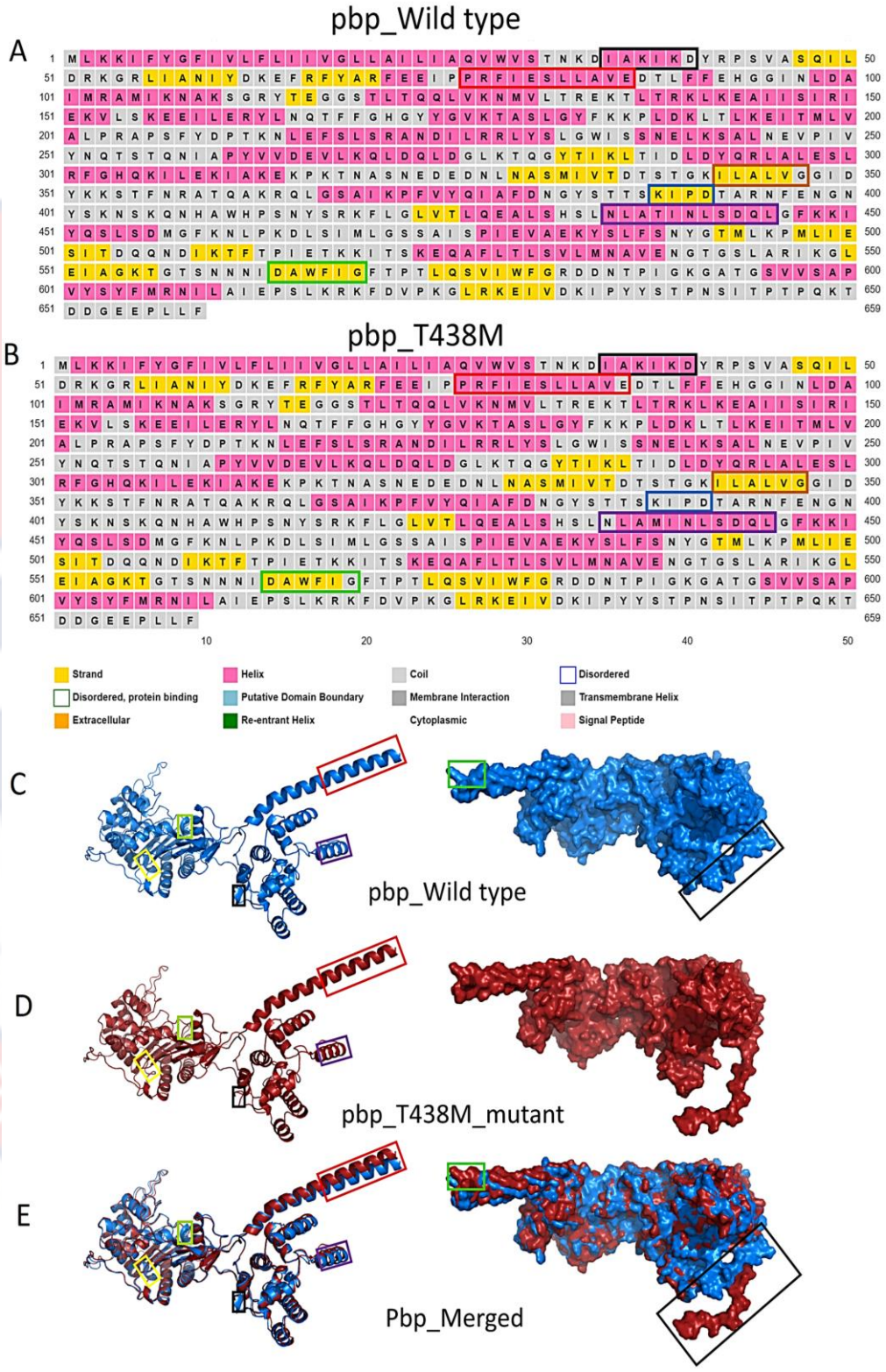


Figure 12: Predicted secondary and tertiary structures of wild type and T438M mutant

Alignment, Annotation of Mutations on Protein Domain and Prediction of both Secondary and Tertiary Structures of Wild Type and Mutant *rdxA* Gene

Nucleotide substitutions (area shown with black rectangles in A) were observed when sequenced genes were compared to reference *rdxA* genes (Figure 13). Areas put in black rectangles show that all samples had nucleotides that were different from the references (shown in A). Annotation of variants indicate that mutations were within the nitroreductase domain of the protein (shown in B) (Figure 13).

Secondary structures generated for wild type (A) and E27G mutant (B) show no clearly distinct areas of modification in the cartoon structures (Figure 14). Slight changes are observed in the surface structures indicated with black rectangles and red squares in C – E (Figure 14).

An area of helix (indicated with red square) in the cartoon of merged structures in the wild type (A) and Y71F mutant (B) show an area of non-alignment (Figure 15). Their corresponding surface structures also show minimal structural modifications (indicated with black and red rectangles). Secondary structures (shown in A and B) demonstrates elongation of helices (indicated with red and blue rectangles) while a longer helix observed in the wild type (A) is replaced with a shorter length strand observed in B and shown with a black rectangle (Figure 15).

Secondary structures generated for wild type (A) and R90G mutant (B) showing an area of a shortened helix (shown with black) part of the deleted region is replaced with a strand (Figure 16). Helices are also further shortened (shown with blue rectangle) or deleted (indicated with green square). In the tertiary structures (shown in C to E), differences are shown with a yellow square (Figure 16).

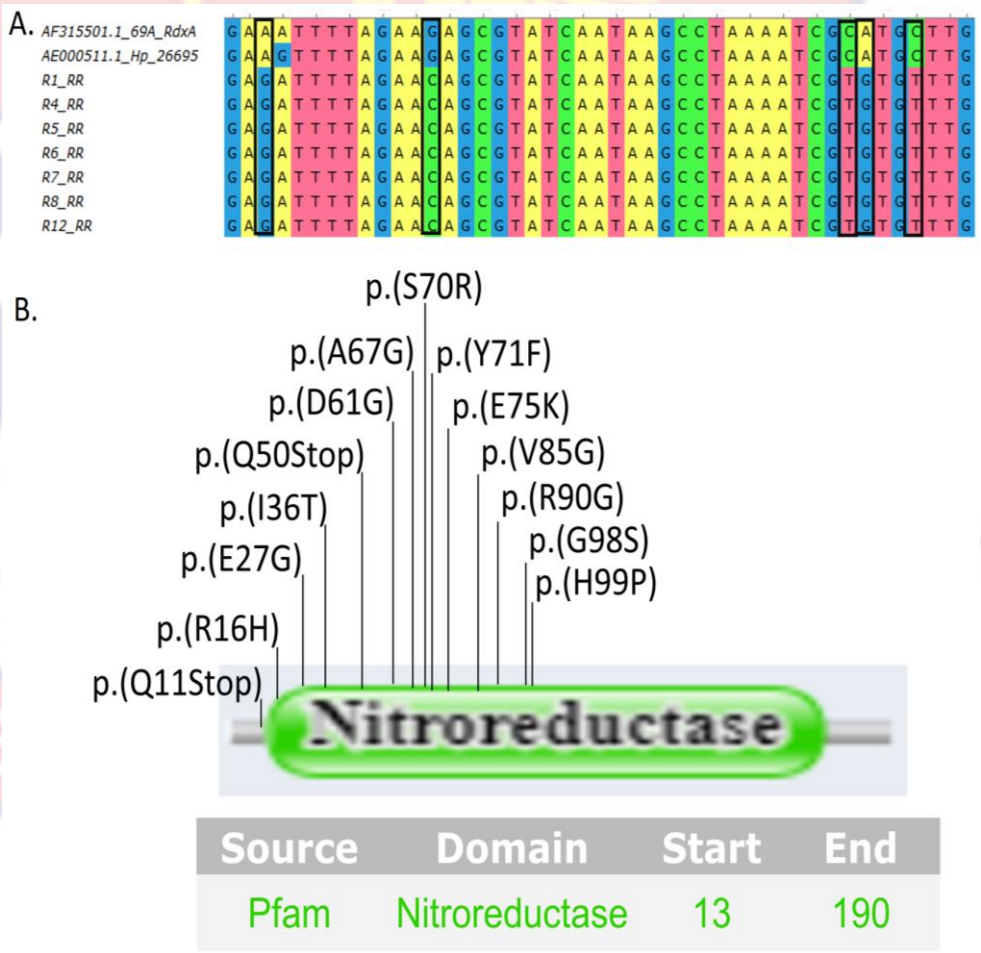


Figure 13: Sequenced *RdxA*-reference strain alignment and annotation of mutations on protein domain

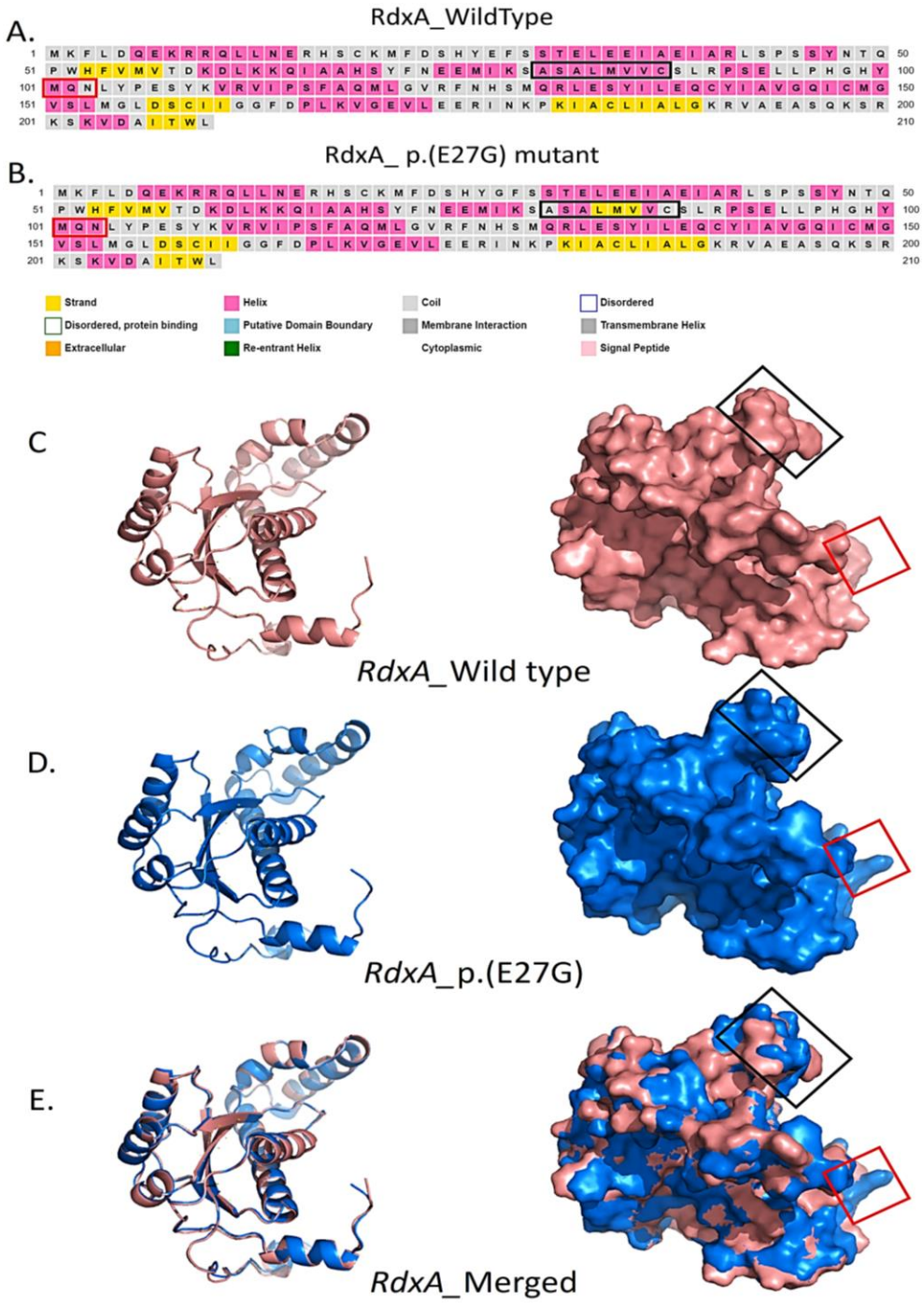


Figure 14: Predicted secondary and tertiary structures of wild type and E27G *RdxA* mutant

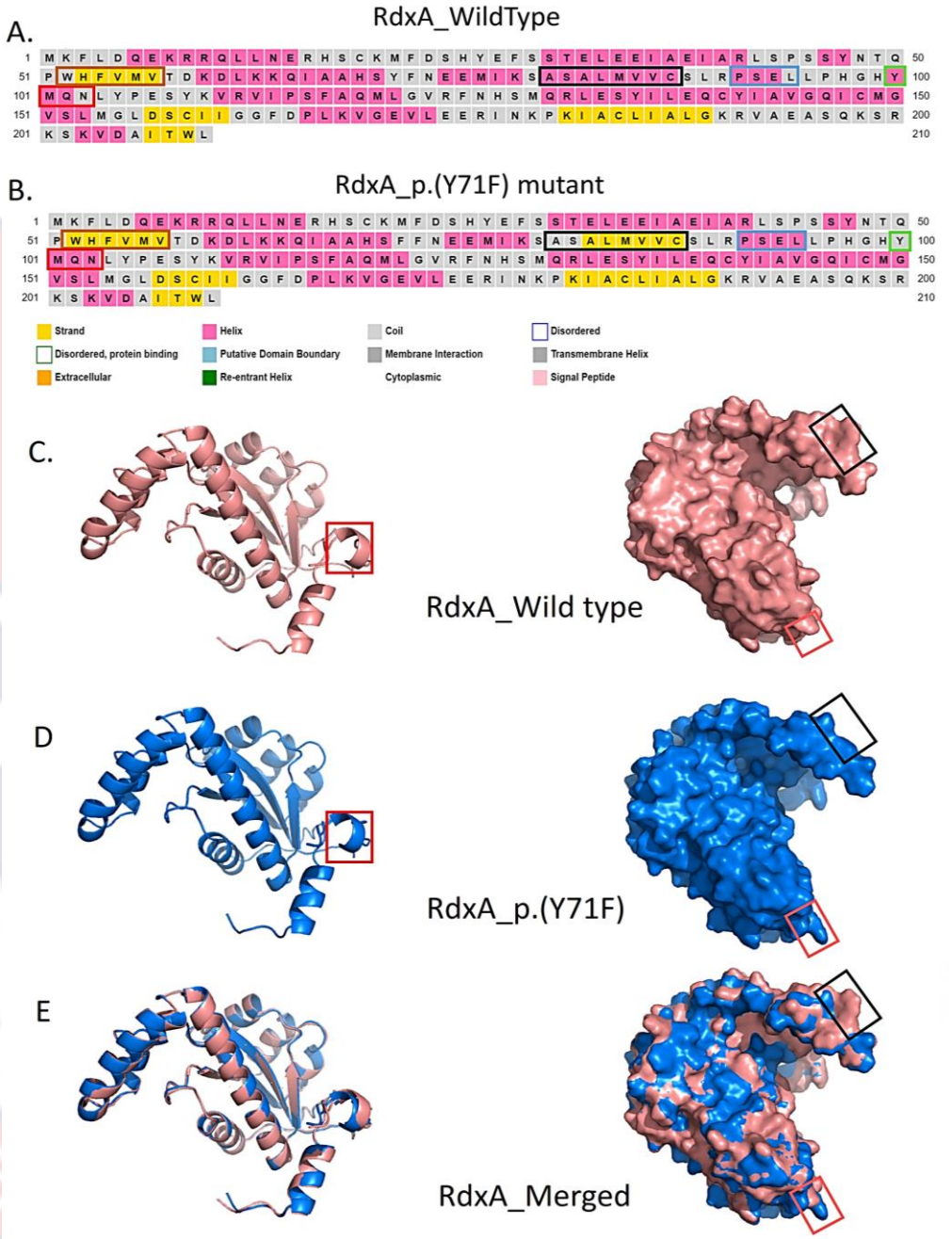


Figure 15: Predicted secondary and tertiary structures of wild type and Y71F *RdxA* mutant

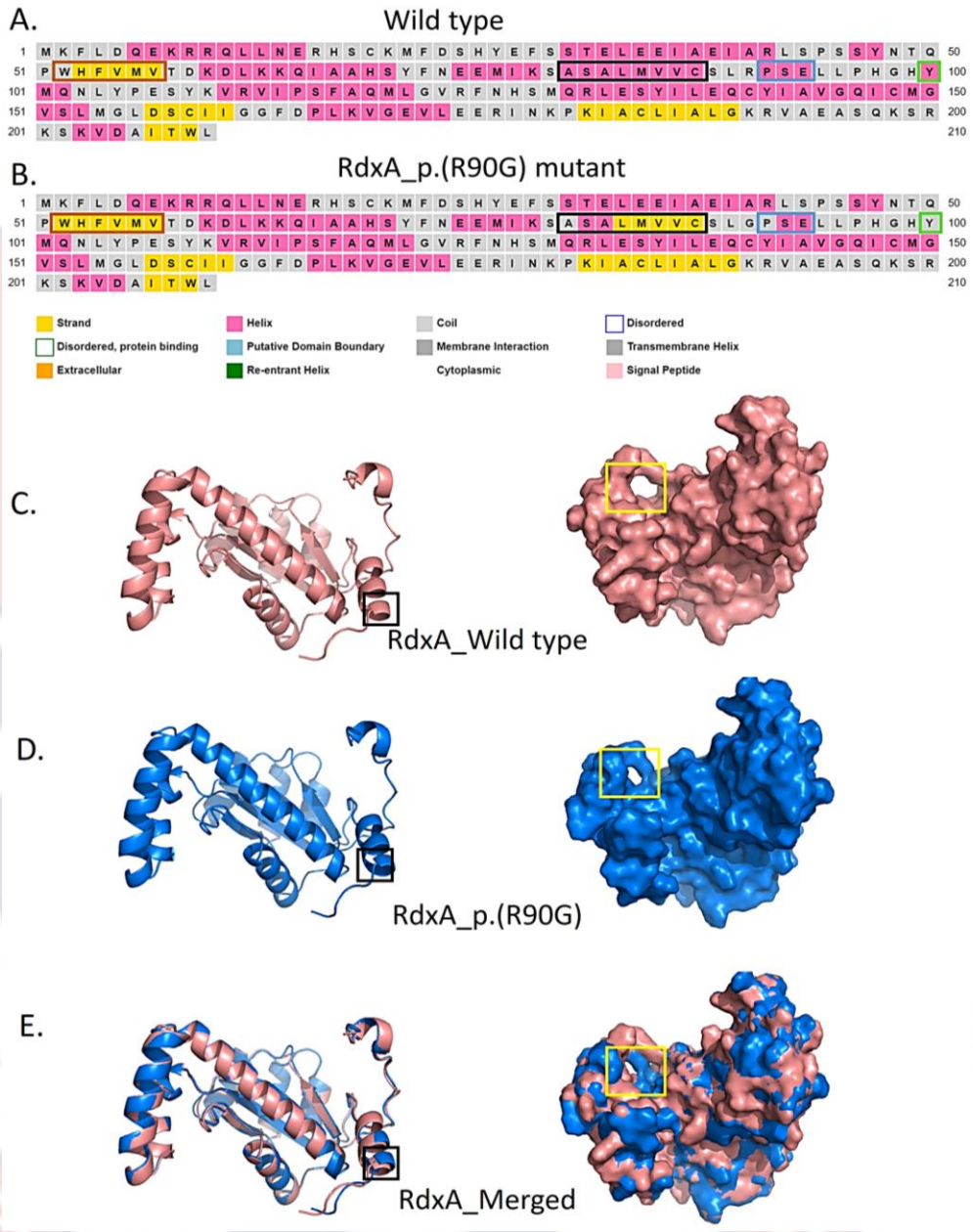


Figure 16: Predicted secondary and tertiary structures of wild type and R90G *RdxA* mutant

CHAPTER FIVE

DISCUSSION, SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Discussion

Reports on infection with *H. pylori* in Ghana suggest high prevalence across different localities. This however has not translated into the level of investigations that merits an infection with such magnitude of prevalence. Socioeconomic factors, lifestyle and bacterial virulence factors have not been extensive enough to unravel this infection which continues to be a burden on many health facilities. As far as literature search is concerned, this is the first report of the detection of bacterial factors such as *cagE* and *dupA* and their association with disease conditions. Again, there are no reports to support the efficacy of the current therapeutic approach targeting this infection. The present study therefore seek to provide information on these factors and throw more light on the situation of *H. pylori* infection in the Central Region of Ghana.

Dynamics of *H. pylori* infection prevalence between the years 2012 and 2019

Information regarding the health burden posed by *H. pylori* infection is widely known and some studies have demonstrated a high prevalence from several countries in the West African region including Ghana (Ofori et al., 2019). While the worldwide rate is found to be around 44.3% for industrialized countries, higher rates are recorded for developing nations (Ahmed et al., 2007; Perez -

Perez et al., 2004; Yuan et al., 2022). The current research is expected to represent the first report on the dynamics of *H. pylori* infection prevalence in the Central Region of Ghana. It is projected to serve as an added information to what is existing elsewhere in the country with the hope of broadening the scope of investigation of the prevalence and the dynamics of associated pathological conditions countrywide. Again, majority of existing reports on the state of the infection in Ghana have depended on unsubstantial sample sizes. For instance, two separate studies by Archampong et al in 2015 and 2017 obtained a prevalence of 74.8 % and 71.1 % when sample sizes of 242 and 159 respectively, were used (Archampong et al., 2017; Archampong et al., 2015). In a different study, 300 records were used by Afihene et al. to obtain a prevalence of 58.72 % (Afihene et al., 2014). A significant variation in prevalence across various studies is observed and this may be linked to the relatively smaller sample sizes. The sample sizes may represent a challenge in making generalized deductions and predictions hence the need for a study with larger numbers.

In addition to the problem of sample sizes, the predominant study areas of *H. pylori* research in the country have been in the capital city of Accra. *H. pylori* infection is however considered a disease of the poor owing to its association with poor sanitary conditions and lower socioeconomic status. Therefore, studies from a region like the Greater Accra of Ghana where conditions are considered better than that of the Central Region cannot produce a true picture of the burden of infection in the country. Analysis of the poverty situation in Greater Accra region has stated a rate of 13.5 % in 2005/06 which reduced to

2.5 % in the 2016/17 data. In the Central Region however, the rate was recorded to be 13.8 % in the 2016/17 data from an initial 23.4% in 2005/06 (GSS, 2018). Clearly, a greater likelihood of transmission attributable to prevailing risk factors is more shifted towards the Central Region.

The current study sought to obtain the dynamics of infection from this bacterium and ascertain the differences in prevalence among males and females, age groups, and how the infection has been over the past few years in the region. To do this secondary data existing in two health facilities who were the main endoscopy referral units in the region were accessed. Past patient records from 2012 to 2019 were obtained and analysed. This provided a relatively larger sample size as compared to other studies in the country to produce a better overview of the disease situation in the region. Out of 1767 patient records which were selected after applying the exclusion inclusion criteria, 1336 records representing 75.61 % were positive for the infection. This was translated into 77.09 % (572/742) for males and 74.54 % (764/1025) for females (Figure 1). The difference in prevalence for males compared to females was not statistically significant ($\chi^2 = 0.026$, $p > 0.05$). The overall prevalence of 75.61 % (Figure 1) is similar to findings obtained from other parts of the country (Archampong et al., 2017; Archampong et al., 2015).

A higher proportion of the participants (1025 out of 1767) were females and this follows the trend in other Ghanaian studies (Adu-Aryee et al., 2016; Afihene et al., 2014; Darko et al., 2015) as well as outside Ghana (Aminde et al., 2019; Toscano et al., 2018). This is particularly so because the diagnosis of functional

gastrointestinal disorder which is usually present as dyspepsia is common in women (Ahlawat et al., 2006). Reports on prevalence from studies in Ghana that are over a decade old such as the findings by Aduful et al in 2007 and that of Barko et al in 1996 indicates no improvement in the burden of the infection for over a decade now. They obtained 75 % and 75.4 % rates respectively (Aduful et al., 2007; Baako & Darko, 1996). Studies from other African countries have comparable results to the current study (Diomande et al., 1991; Tadesse et al., 2014). The high prevalence is also similar to those of the Middle East and other developing geographical regions of the world where favorable conditions such as poor sanitation, overcrowding conditions and low socioeconomic status are existing (Toosi et al., 2021; Yuan et al., 2022).

This study looked at the trend in prevalence from 2012 to 2019 (Figure 2). It was revealed that the estimated prevalence was generally high throughout the years under review though fluctuating in some years. This worrying situation may suggest prevailing adverse environmental and socio-demographic conditions that may be promoting infection. This fluctuating trend is similar to those reported in other studies in Africa (Marrone et al., 2014; Workineh & Andargie, 2016). In Iran, a constant trend was achieved in a 5-year report from 2010 to 2015 (Salehi et al., 2017). In the present study, the generally high prevalence was not only found across all years under study but was also found to be at least 72 % for each of the age group categories (Figure 3). This suggests that the burden of the infection is not peculiar to an age group and has maintained a high prevalence for several years.

Relationship between *H. pylori* infection and gastro-duodenal pathologies

There are several digestive illnesses that are predisposed to by an *H. pylori* infection. *H. pylori* infection has been linked to an increased incidence of stomach cancer and peptic ulcers by 2% and 20%, respectively (Kusters et al., 2006). The persistence of infection which can exist throughout an individual's life comes with varying degrees of host response depending on certain factors. Elements such as age, gender, lifestyle, bacterial genetic factors among others have a role to play in the degree of infection rate. This in turn causes a variation in related ailments across different locations of the world. What this brings is a situation where a single country generates wide variations in prevalence in different regions of its land space. This adds to the need for the sub region of Central Ghana to have an idea of the infection situation or burden. Pathological outcome in relation to infection in this study was found to be proportionally higher as compared to other Ghanaian studies such as those by Afihene et al. (2014) and Darko et al. (2015). These past studies observed the absence of pathological conditions among infected subjects up to about half of their study population. Judging from the poverty situation in the Central Region of Ghana, it is very likely that a lot of people may delay their visit to health facilities when symptoms set in until their condition deteriorates because of monetary issues. That is, only patients who have severe or persistent symptoms get motivated to mobilize the scarce resources available to undertake the investigation. This may

be the reason for higher pathological situations found in the current study compared to those of Darko et al and Afihene et al.

Pathological onset in infections of *H. pylori* is characterized by inflammatory responses that contribute to persistence and final disease manifestation. Colonized areas of infection are associated with influx of T helper (Th) lymphocytes such as Th1, Th17, Th22 and T regulatory (Treg) cells. There is also an increase in cells such as IL-10^{+}, $CD19^{+}$, and B cells, which are noted to play key roles in the establishment of *H. pylori* infection pathogenesis (Nagini, 2012; Olokoba et al., 2013; Wiredu & Armah, 2006). For instance, it has been established that in *H. pylori* infected functional dyspepsia individuals, increased Th17 T-helper cells is an indication of an increased risk of chronic gastritis (Nyarko et al., 2005; Singh et al., 2020). Again, in such individuals, the higher levels of IL-10^{+}, $CD19^{+}$, and B cells signifies a possible onset of gastritis compared to levels found in persons with *H. pylori* related PUD patients (Nahid-Samiei et al., 2020; Wiredu & Armah, 2006).

In the current study, gastritis and esophagitis were revealed to be significantly associated with *H. pylori* infection after analysis of the secondary data. Patient records that were positive for the infection had about four-times more the tendency to develop gastritis and three-times more the likelihood for the development of esophagitis (table 2). Among the various age groups, esophagitis and gastritis recorded an increase in prevalence with increasing age until at age group of >70 years when it reduced (table 3). The prevalence of these two conditions (61.53% and 30.84 % for gastritis and esophagitis

respectively) were significantly associated with infection. Though not significant as compared to this study, gastritis have been found to be the highest *H. pylori* associated gastroduodenal pathologies from other studies in Ghana (Aduful et al., 2007; Afihene et al., 2014; Agyei-Nkansah & Amoako Duah, 2019). Analysis of data by Darko et al. (2015) found gastritis as the third highest (8.1 %) condition in 1999 but appeared the highest condition recording a rate of 50.2 % in 2015. The diagnosis of esophagitis has been recorded at low rates (5.3 – 8.0 %) in areas like Kumasi and Accra (Aduful et al., 2007; Afihene et al., 2014) compared to the 30.84% revealed in this work. The report in the Northern region of Ghana showed no statistically significant association between *H. pylori* and gastritis (Tabiri et al., 2016). Other parts of the African continent obtained a similar positive association between *H. pylori* and gastritis (Diomande et al., 1991; Oyekale, 2017).

When a person gets infected with *H. pylori*, the related gastric mucosal inflammations can cause the mucosa's natural structural integrity to be lost (Kusters et al., 2006). In a chronic inflammatory disease known as atrophic gastritis, such anatomical changes are typically followed by destruction to the stomach glands and replacement by fibrosis. According to reports, the degree of atrophy in people with *H. pylori* infection increases their likelihood of developing gastric cancer (Kusters et al., 2006). Therefore, the high incidence of *H. pylori* related gastritis if left untreated could increase the risk of development of gastric cancer. Increased observation of abnormal endoscopic finding may be as a result of changes in prevailing risk factors for transmission.

Gastric ulcers and hiatal hernias (HH) were discovered to be strongly related to *H. pylori* infection. These were the two conditions that followed in degree of prevalence after gastritis and esophagitis (table 2). The development of hiatal hernia is influenced by several factors, including intra-abdominal pressure, obesity and age (Pawluszewicz et al., 2018). HH has been found to be more prevalent in older people and this may be due to muscle weakness and decreased elasticity (Pawluszewicz et al., 2018). The observation of generally higher frequencies of HH diagnosis with age (table 3) may therefore be due to these factors associated with old age. A rather reduced rate for >70 years may be as a result of the relatively smaller sample size (table 3). Esophagitis is strongly associated with HH such that the frequency of the incidence of cases of symptomatic HH is often synonymous with the existence of esophagitis (Pawluszewicz et al., 2018; Petersen et al., 1991). The increased prevalence of esophagitis diagnosis with HH may therefore be due to this relationship. With the exception of age group < 20 years, all groups recorded at least 20% diagnosis of esophagitis similar to observations made for HH and they generally increased with increasing age (table 3).

Though not significant, the striking observation in the prevalence of esophageal and gastric cancers was their higher association with males and old age. There were nineteen (19) gastric cancer patient records and out of this, 16 were males and 3 were females (Figure 4). All six (6) esophageal cancer patients were males with 3 of them being over 70 years of age (Figure 4 and Table 2). The remaining three (3) were between the ages of 41 and 70 years. Fifteen (15) out of the

nineteen (19) gastric cancer patients were over 51 years (table 3). Worldwide, gastric cancer is considered a disease of the elderly with the multitude appearing in men. This is further revealed in a 10-year review of hospital mortality and autopsies in Ghana in which gastric cancer cases showed relative frequencies of 6.3 in men compared to 3.6 in females (Wiredu & Armah, 2006). The study also reported a general increase in gastric cancer cases with increasing age which is comparable to the trend observed in the current report and others (Nagini, 2012; Simpong et al., 2018). Salt accumulation together with genetic defects have been considered as other possible explanations for the higher rates in males and the aged (Nagini, 2012). Furthermore, smoking and excessive alcohol intake which are habits largely associated with men have been identified as risk factors for gastric cancer and may be contributing to the higher cancer rates in men (Ito et al., 2021; Ramos et al., 2018; Yusefi et al., 2018). These factors could be the reason for the realization of all esophageal cancer findings in this study being greatly associated with older males. The association of cancer diagnosis and infection were however not significant. With the exception of lax lower esophageal sphincter, all conditions recorded were higher in males than females (Figure 4).

In studies from Ashanti, Northern and Greater Accra regions of Ghana, duodenal ulcer appears to be a very common endoscopic pathology which is different from the observation made in this study. A rate as high as 72 % has been identified in the Northern region (Tabiri et al., 2016), 10-20 % in Accra (Adu-Aryee et al., 2016; Darko et al., 2015) and 10.6 % in Kumasi (Afihene et

al., 2014) compared to the 4.04 % in this study (table 2). This is, however, similar to the findings in Akwatia where a 5.4 % rate was obtained (Agyei-Nkansah & Amoako Duah, 2019). Factors such as the possible existence of an *H. pylori* strain that is different from what exists in other parts of the country, co-infection by multiple strains, and changes in nutritional requirements may be contributing to this wide variation (Gu et al., 2019; Lee & Van Zanten, 1997). Nonetheless, duodenal ulcer was found to have a significant association with *H. pylori* infection. There were several cases of more than one condition recorded for a single person. With the exceptions of portal hypertensive gastropathy 52.94 % (9/17) and retching gastropathy 33.33 %, (1/3), all other diagnosis had more than 65% of affected individuals testing positive for *H. pylori*; the highest being those with gastric cancer (19 out of 21 records representing 90.48 %) followed by duodenal ulcer (54 out of 60 records representing 90 %).

Risk factors are associated with *H. pylori* infection prevalence

Analysis of socioeconomic factors was done on the 169 participants who consented to partake in the study. After patients had been enlightened on the nature of the study, questionnaires were administered. At the end of the entire procedure, the answered questionnaires were retrieved and analysed using GraphPad prism version 8. The results of PCR for the detection of *Hp23Sr6*/*r7* which signifies the presence of *H. pylori* were relied on for positive samples after rapid diagnostic test results from the facilities showed some inconsistencies with PCR findings. All 169 patient biopsies were taken through PCR for detection of *H. pylori* colonising the antrum of the stomach.

H. pylori colonizes multiple hosts from a variety of environmental sources including blood, faeces, dental plaque, and water (Isaeva & Isaeva, 2020). From these sources, transmission to individuals is usually through oral-oral and faeco-oral. The frequency of exposure to these factors is directly linked to lower socioeconomic status where factors such as place of early life, poor sanitary and hygiene practices, and net income contribute to prevalence of infection. In the current study, number of persons sharing accommodation (p value = 0.6282), income (p value = 0.0712), and place of early childhood stay (p value = 0.8061) were not statistically linked to *H. pylori* infection. However, the level of education (p value = 0.0017), marital status (p value = 0.0002), and job status (p value = 0.0154) had a significant relationship with infection. A similar study in Ghana showed a significant association with persons sharing accommodation, marital status, and source of drinking water but not for level of education (Awuku et al., 2017). Educational level showed an inverse relationship with infection in a study by Kotilea et al. (2019). The effect of the source of drinking water in this study was statistically significant (p value = 0.0142). In some studies elsewhere, it was realised that having parents who are infected and bed-sharing remained two factors that contributed to increased prevalence *(Hasosah et al., 2015).* Primarily, the developed world has better economic conditions and higher socioeconomic status which is yielding decreasing prevalence. Conversely, higher rates are recorded in lower to lower-middle income countries where prevailing factors contribute to the spread of infection (Hasosah et al., 2015; Kotilea et al., 2019). In Japan, the prevalence in children between the ages of 12-15 have recorded as low as 3.1 to 4 % while

a review in Poland covering the years 2000 to 2013 has reported rates within the range of 8.9 % and 23.1 %. The existing adverse hygienic conditions in a relatively poverty ridden region like the Central part of Ghana where data for this study was resourced explains the increasing prevalence.

Differences in rate also occur among people of different ethnic group even within the same country. This is evidenced by a study that reported over 60% infection rate for Mexican Americans compared to 30% for non-Hispanic white population in the same country (Grad et al., 2012). Data analysed in this study had most of the participants being of the same tribe. Majority of the participants were either Ashantis or Fantes. While the presence of spicy foods wasn't related, the frequency of vegetable intake had a direct relationship with infection. In a study involving 166 adult Portuguese subjects, a higher prevalence of infection was identified among participants who, in addition to their preference for fried foods, consumed less vegetables/fruits. Meanwhile, *H. pylori* has been successfully isolated from raw vegetables and salad making them possible means of transmission (Kotilea et al., 2019; Yahaghi et al., 2014). This study reports a significant association with the intake of vegetables which could be as a result of a possible transmission through these food materials as identified in previous studies including that of Kotilea et al.

There was no association between alcohol intake and smoking as observed in other studies in Ghana and elsewhere (Tabiri et al., 2016; Zhu et al., 2014). Diet restrictions like non-acidic foods and boiled vegetables help in digestion and bowel function. Others like green tea, antioxidant –rich diet and other dietary

habits may help reduce risk of infection (Nabavizadeh et al., 2022; Rueda-Robles et al., 2021). There was a significant relationship between infection and diet restrictions as well as a personal or family history of dyspepsia in this study just as recorded in others (Kouitcheu Mabeku et al., 2018). The nature of diet restrictions explained by participants were mainly about eating times. Other studies found no association with a history of dyspepsia (Shokrzadeh et al., 2012). Analysis of risk factors is an important area in understanding reinfection pathways especially after successful eradication. Such factors are suspected to be involved in relapse and infection recurrence. It is therefore important that eradication therapeutic measures be as accurate and effective as possible and relapses properly treated after primary eradication.

Association of *H. pylori* virulence genes with gastric diseases

Epidemiological and basic research demonstrate that infection with *H. pylori* strains carrying particular virulence factors can result in more severe outcomes than other infections. The virulence of the infecting strain plays a significant role in the progression of the disease in those who are *H. pylori* positive. Some bacteria, like *H. pylori*, have characteristics known as virulence factors that make them more prone to spread disease than other pathogens. If sufficient evidence can support this claim, it can be concluded that not all *H. pylori* strains are harmful. It has been shown that all strains of this bacterium are capable of causing a long-lasting histologic gastritis with lymphocytic and mildly neutrophilic infiltrate, yet this inflammatory response is clinically undetectable. However, it is well recognized that some strains have significantly higher risks

of cancer and stomach ulcers than others. Certain traits and properties are only present in some strains and are associated with disease making them potential therapeutic targets and an important research target as well. Out of the 169 patients whose biopsies were analysed by PCR for the presence of *H. pylori,* 103 were positive for the bacterium. The positive samples were investigated for the presence of bacterial virulence factors and their possible association with disease outcome. Several bacterial and host factor interactions determine the development of clinical manifestations of *H. pylori* infection. In this study, the frequencies of genotypes such as *dupA*, *iceA2*, *cagE*, and *vacA* subtypes including *s1*, *s2*, i1, *i2*, *m1*, *m2*, were determined. Their association with pathology was also analysed. There was no successful amplification of *s1*, *s2*, and *i1 vacA* subtypes.

dupA gene was the most prevalent virulent factor identified in the studies. It occurred at a rate of 32.04% followed by *iceA2* gene which recorded 28.16 % (table 5). It was also found to occur as a single genotype at 18.45 % but together with other genes as well (table 6). There are conflicting results on the role of *dupA* in disease outcome. However, a number of studies have found *dupA* to have a significant relationship with duodenal ulcer and a negative association with gastric cancer (Karbalaei et al., 2021; Lu et al., 2005). This has been the case in findings from countries such as Japan, South Africa, North India, Brasil, South Korea, and Colombia and in majority of parts of East Asia and South America (Alam et al., 2020). In contrast, some studies found no association (Argent et al., 2007; de Lima Silva et al., 2021). To the best of our knowledge,

this is the first report on the detection of *dupA* gene of *H. pylori* in Ghana. Even though *dupA* gene was largely presented in the study subjects, analysis of findings showed no significant association with pathological condition. This may suggest no direct relationship between *dupA* and clinical condition in Ghana which is comparable to studies elsewhere as described by Alam et al. (2020). It may however, be able to do so in combination with other factors.

There is an unresolved controversy about how the presence of the *iceA* affects clinical outcome in *H. pylori* infection (Essawi et al., 2013). *iceA* is a gene that is up-regulated when *H. pylori* comes into contact with host epithelium. While some studies found a close association, others didn't (Essawi et al., 2013; Proença-Modena et al., 2009a). Some findings have indicated that this gene is inversely associated with gastric ulcer disease (Shiota et al., 2012). The discrepancies to the prevalence of either of the two allelic variants *iceA1* or *iceA2* is equally wide spread. *iceA2* was recorded as the most prevalent virulent factor in a study by Essawi et al. (2013) and this is similar to the detection of *iceA2* as the second most prevalent in this study (table 5). Analysis of the two alleles in a single study has produced different rates at different geographical locations. For instance, In East Asia, the *iceA1* genotype is reported to be predominant (76%), whereas *iceA2* is predominant in Portugal and Colombia (Wu et al., 2005). In the current study, *iceA*1 was not detected in any of the samples but rather *iceA2* at a rate of 28.16 % (table 5). *iceA2* prevalence has been recorded at 90.1% and 84.4 % in Brasil and Palestine respectively while at a low rate of 9.0 % in Mexico (Essawi et al., 2013). Meanwhile, epidemiological

studies have found a link between *iceA* and duodenal or gastric ulcer development (Kidd et al., 2001). *iceA2* detection in this study shows a significant association with duodenal ulcer (p = 0.0214, OR = 11.7, CI = 1.74 - 144) and gastritis (p= 0.0005, OR = 7.78. CI = 2.27 - 25.70) (table 8). A meta-analysis on western countries has shown an increased risk of peptic ulcer (OR = 1.28) in patients with seropositive *iceA1* (Shiota et al., 2012). Meanwhile, *iceA* gene has been suggested by a number of scientists to be an indication of genetic variability across different geographical locations and not necessarily a sign of an increased risk to a specific gastric disease (Caner et al., 2007; Podzorski et al., 2003; Yamaoka et al., 1999). Perhaps, the detection of only *iceA2* allelic variants in this study is an indication of the type of strain predominant in Ghana which may be different from other studies where the predominant *iceA* gene detected were the *A1* type.

cagE and the *m2*, *i2* allelic variants of *vacA* were identified to have no significant relationship with clinical conditions (tables 9, 11, and 12 respectively). *cagE* is reported to be a more accurate marker for defining the complete presence of *cagPAI* (Khatoon et al., 2017). In places like Turkey and India, *cagE* has been linked to GC (Lima et al., 2011). Other studies found no association with pathology (Dabiri et al., 2017; Dadashzadeh et al., 2017). *i2* and *m2* subtypes of *vacA* showed no association with pathology as is the case in several studies (Mandour et al., 2021; Monroy et al., 2022; Vital et al., 2022). In Ghana, Archampong et al. (2017) observed the presence of *vacAm1* genes in 44.7 % of isolates compared to 22.0 % in *m2* isolates. The genotypes in that

study had no significant association with clinical conditions. In the present study, the *vacAm1* allelic variant was identified to be associated with gastric ulcer (p = 0.001, OR = 15.9, CI: 3.15 - 64.50), duodenal ulcer (p = 0.0029, OR = 27.9, CI = 4.44 - 168) and varices (OR = 31.3, CI = 3.01 - 454) (table 10). Apart from *vacAi2* and *dupA2* genes which were identified to have occurred at higher frequencies in females than males, all other virulent factors were more prevalent in males. There were cases of multiple occurrence of genotypes (table 6) including a 2.91 % each of *cagE*/*dupA* and *iceA2/dupA/vacAi2*. *iceA2/dupA* appeared in five (5) subjects indicating a 3.88 % prevalence. The mid-region of *H. pylori vacA* is variable and appear as either m1 or m2. Analysis of the *vacA* mid region of this study revealed situations where both regions were detected from individual samples. From table 6, *m1* and *m2* were observed to have been amplified together in some study subjects. Some subjects had only these two subtypes (*m1* and *m2*) while others harboured the two together with other genotypes. In all, *m1* and *m2* subtypes occurred at the same time in four (4) samples (table 6). This is a clear indication of cases of mixed infections. Multiple infections with genetically diverse bacteria, especially mixed infections involving antibiotic-susceptible and resistant isolates, are difficult to detect for effective treatment. This compromises the efficacy of eradication therapies. Multiple infections are widely believed to be more common in developing countries which makes the current detection not surprising. Multiple infections has been detected in other study subjects with some measuring as high as 85 % (Mansour et al., 2016; Sheu et al., 2009). Mixed *H. pylori*

infections exhibit high genetic diversity and may facilitate bacterial adaptation to the hostile gastric environment and contribute to disease development.

Among the total participants (N=103) of the study, there were 40 males and 63 females. The detection of virulent factors were commoner in males than females for most of the factors under study. Both *iceA2* (42.50 % in males and 19.05 % in females) (table 5) and *vacAm1* (15 % in males and 3.17 % in females) (table 5) which recorded significant association with various diseases (tables 8 and 10) were found to be more in males, it indicates that the likelihood of adverse condition as a result of infection will be more probable in males than their female counterpart.

Antibiogram of *H. pylori* infection among patients presenting with the various gastroduodenal pathologies in Central Region

Effective treatment of *H. pylori* infection is largely achieved when antibiotics are combined in a single therapy. The combination is also to reduce the chances of antibiotic resistance (Pohl et al., 2019). Infections caused by peptic ulcerative illness can be effectively treated to not only alleviate gastritis but also stop them from spreading and recurring. Effective eradication will eventually lower the chance of getting stomach cancer, which will cut down on the expense of treating more serious *H. pylori*-related diseases down the road. Amoxicillin, tetracycline, or clarithromycin, along with metronidazole, a proton pump inhibitor (PPI), or bismuth salt, are the three medications used to treat *H. pylori* infection (Suzuki & Mori, 2018; Suzuki et al., 2010).

In Ghana, just like other unindustrialized countries, *H. pylori* has infected the majority of the adult population (Archampong et al., 2017; Ofori et al., 2019). Treatment of *H. pylori* infection in Ghana according to the Standard Treatment Guidelines (*GNDP*, 2017), involves a combination of any two of amoxicillin, clarithromycin, and metronidazole plus a proton pump inhibitor. As far as literature search is concerned, there are no studies in Ghana aimed at ascertaining the efficacy of any of the recommended antibiotics against *H. pylori* infection. This may imply that the current treatment regimen may have been applied on the basis of studies from elsewhere which may not be very effective against the strains present in Ghana. Numerous studies have shown that the distribution of *H. pylori* varies not only from one country to the next but also within the same country's various regions (Vilaichone et al., 2013). Again, variations exist in strains and their characteristics across different locations which also affects the pathologies that may be presented (Grad et al., 2012; Xue et al., 2021). All these point to the fact that the basis for a choice of treatment of *H. pylori* infection in a particular geographical location has a greater chance of being the wrong choice if applied to a different place.

Genotypic antibiotic resistance pattern among *H. pylori* positive patients

In this work, all 103 *H. pylori* positive samples were investigated for the presence of antibiotic resistant genes. This was done by PCR amplification of *gyrA* and *gyrB* for fluoroquinone drug resistance. Fluoroquinone dugs used in the study included ciprofloxacin and levofloxacin. Primers designed with substituted residues including *tet-1* for tetracycline resistance was also

investigated. The presence of resistance gene for amoxicillin (penicillin antibiotic) was also analysed using pbp1 gene for all 103 positive samples. The highest percentage antibiotic resistant gene was obtained for fluoroquinone drugs (ciprofloxacin and levofloxacin) where 67.96 % of the total samples were positive for *gyrA* but no detection of *gyrB* resistant gene (table 13). This is the first report of antibiotic resistance of *H. pylori* against to fluoroquinolones in Ghana and results are comparable to the rate of 55.7 % in China but very high compared to a range of 2.8 – 20 % areas such as Korea, Southern Taiwan, Japan, and Italy (Wang et al., 2010). Metronidazole resistance followed in prevalence when a rate of 45.63 % was observed for the amplification of *rdxA* resistance gene (table 13). Resistance to metronidazole was observed to occur together with co-resistance (multiple resistance) to amoxicillin at a rate of 4.85 % and to *gyrA* for quinolone resistance at a rate of 14.56 % (table 13). Patients with triple resistance indicated by the amplification of *rdxA*, *gyrA*, and *pbp1* resistance genes occurred at 16.50 % (table 13). Multiple resistance has been reported in extensive review investigations in Africa and elsewhere (Jaka et al., 2018; Kasahun et al., 2020; Kouitcheu et al., 2019). Amoxicillin, which remains one of the antibiotics prescribed in the triple-therapy regimen against this infection in Ghana, showed 35.92 % resistance indicated by the successful amplification of *pbp1* resistant genes (table 13). There was no detection of tetracycline resistant genes among all samples processed. Resistance to amoxicillin isn't as widespread as that of metronidazole and fortunately, a lot of studies have reported low resistance rates to amoxicillin and tetracycline (Diab et al., 2018).

In Nigeria, a rate as high as 100 % have been observed of *H. pylori* against amoxicillin (Aboderin et al., 2007).

From table 14, the presence of *rdxA* gene for metronidazole resistance was represented at higher percentages in most of the disease conditions. All gastric cancer cases had this resistant gene. Metronidazole is widely used in many common infections and therefore has a bigger chance of resistance. It was observed to be present at a rate of 21 – 40 % in gastritis, duodenal and gastric ulcers, and duodenitis cases. The lowest percentage presence of the *rdxA* gene (13.0 %) was found in gastro-oesophageal reflux disease (GERD). Fluoroquinone resistance identified by the presence of *GyrA* resistant genes appeared to occur at high rates (at least 40 %) in all disease conditions. This shows that strains with this resistant gene produce diverse disease conditions from more severe to mild states. *pbp1* was not detected among duodenitis and gastric cancer cases. This gene was present in 13.8 % and 28.6 % cases of gastritis and gastric ulcer cases respectively.

Phenotypic antibiotic susceptibility and resistance against antibiotics used in first-line treatment of *H. pylori* infection

After all positive samples had been taken through the genotypic detection of resistance by amplifying resistant genes, 15 cultured biopsy samples which were the successfully cultured samples were taken through phenotypic antibiotic susceptibility testing. In this procedure, resistance to Levofloxacin (5 µg), Tetracycline (30 µg), Ciprofloxacin (5 µg), Metronidazole (5 µg), Clarithromycin (15 µg), amoxicillin (10 µg), and amoxicillin-clavulanic acid

(30 µg) contained in an Oxoid 6-mm-diameter disks were determined in isolates of *H. pylori* by disk diffusion. Amoxicillin-clavulanic acid combination was added because it has been reported to produce higher inhibitory activity against *H. pylori* infection in some areas (Alrabadi et al., 2021; Dehghani et al., 2009; Ojetti et al., 2004) and hence tested for a possible breakthrough therapy in Ghana. The results unfortunately shows a 100 % resistance to amoxicillin-clavulanic acid.

There was complete phenotypic resistance (100 %) to four (4) of the antibiotics under study namely metronidazole (MTZ), amoxicillin (AMX), clarithromycin (CLR) and amoxicillin-clavulanic acid (AMC-CA) (table 14). CLR, AMX, and MTZ constitute the three (3) antibiotics outlined by Ghana's Ministry of Health's GNDP for treatment of *H. pylori* infection. These are prescribed to be used in combination with a proton pump inhibitor. This study may therefore serve as grounds for further scrutiny and a possible second look at the antibiotics of choice against this infection in Ghana. The findings indicate that standard triple therapy comprising of MTZ with AMX or CLR should not be considered as first-line treatment. These findings are comparable to studies such as those by Kouitcheu et al. (2019) and a review of the resistance situation in Nigeria by Chukwudike et al. (2021). In a review article on the average resistance rate of antibiotics to *H. pylori* from different parts of Africa, the rates of resistance to amoxicillin, tetracycline, clarithromycin, and metronidazole were 72.6% (95% CI: 68.6-76.6), 48.7% (95%CI: 44.5-52.9), 29.2% (95%CI:26.7-31.8), and 75.8% (95% CI: 74.1-.77.4) respectively (Jaka et al., 2018). This indicates a

high prevalence in developing countries which could impair the first line triple therapy of infection. This also means there is the need to conduct surveillance of *H. pylori* susceptibility pattern in Africa to determine the extent and nature of dual and triple resistance to aid in empirical treatment.

MTZ (17.5%) and AMC-CA (13.4%) are reported to be the top two antibiotics prescribed in Ghana basically for community-acquired infections and systemic use (Labi et al., 2018). Other studies in Ghana have also reiterated the frequent use of CIP, AMX, AMC-CA, MTZ among the citizenry in a variety of treatment of infections (D'Arcy et al., 2021; Opintan et al.,, 2015). The frequent prescription of these drugs as common anti-microbial agents could be the cause of the high level of resistance in *H. pylori*. Some other factors that might be relevant to this resistance could be mutational inactivation of antibiotic genes of the organism such as mutations in rdxA for MTZ, V domain of 23S rRNA for CLR resistance and within PBP 1-4 region for amoxicillin resistance. Drug resistance during therapeutic application would result from such changes rather than the organism dying. It is therefore proven, in line with earlier research, that LEV, TET, and to some extent CIP may be more effective as first-line *H. pylori* eradication therapies in Ghana than MTZ, AMX, and CLR.

Mutations in *H. pylori* antibiotic target genes and effect of variants on secondary and tertiary structures of proteins

DNA from the fifteen (15) successfully isolated *H. pylori* samples were extracted and amplified for the presence of the target resistant genes using the primers utilised earlier. Sanger sequencing results were then analysed for the

detection of possible mutations in the various genes. Mutations play major roles in the antibiotic activity against bacteria. Antimicrobial resistance is currently recognized as an unavoidable factor in the failures associated with the treatment of *H. pylori* infections in many parts of the world. To this effect, investigation of the antimicrobial susceptibility profile of *H. pylori* is paramount before the most efficient treatment regimen can be obtained. Again, the core of the molecular mechanisms involved in this infection have not been fully understood and researchers continue fill in the loopholes. As far as literature search is concerned, there are currently no available data on the antibiotic profiling including molecular mechanisms of *H. pylori* infection within the Ghanaian population. Although treatment approach is well specified (*GNDP*, 2017), investigations into their efficacy has not been found. The recommended treatment guideline in Ghana suggests a 10-14 day sequence of infection management involving a PPI plus a combination of any two of amoxicillin, clarithromycin, and/or metronidazole (*GNDP*, 2017). This regimen is waning in efficacy resulting in the long standing of symptoms as seen over 66 % of infected individuals in the current study having stayed with their symptoms for over a year (table 4). Hence, there is the need to understand the mechanism of bacterial resistance to the current antibiotics as an important step to eradication of infection.

In the present study, DNA from the fifteen (15) successfully isolated bacterial strains were extracted and amplified for the confirmation of the presence of *H. pylori* and subsequent detection of antibiotic resistant genes namely *RdxA*, *GyrA*

and *pbp* using the primers sets detailed in table 1. These are genes, mutations in which have been identified to contribute to resistance to metronidazole, levofloxacin, and amoxicillin respectively. Amplified PCR products were sequenced and analysed for mutations. Variants were investigated for changes in amino acid sequence in the protein and the resultant mutant proteins were assessed for possible protein structural conformational changes which may be contributing to drug activity failure.

When mutations occur, they can result in modifications of antibiotic target sites and reduced membrane penetrability, affecting the activity of the drug (King, 2021). Mutations in fluoroquinolone (eg. levofloxacin) drug targets in *H. pylori* is mainly by the inhibition of DNA gyrase (topoisomerase II) enzyme encoded in distinct regions such as within the *gyrA* gene. Point mutations in sections of the gene encoding amino acids at positions 86-88, 91, 97, or 130 have been widely associated with levofloxacin resistance (Arslan et al., 2017; Garrido-Treviño et al., 2022; Malfertheiner et al., 2017). As an important alternative drug for the Ghanaian population, analysis of the mutated *gyrA* encoding genes were investigated. Amino acid substitutions were observed at positions H200Y, A199V, A199T, R190S, H189Y and A97V and annotated in the figure which illustrates the domain of the protein. However, none of the variants was identified more than once. Phenotypic observation showed a 40 % resistance to levofloxacin which may be responsible to the low detection rate. Among the variants, A97V and H189Y were found in resistant strains while the remaining were all susceptible to levofloxacin. These variants are identified within the

topoisomerase-primase (Toprim) and in the breakage reunion domain of gyrase (Figure 15C).

Further analysis of mutants was based on the identification of variants in a conserved region of the protein sequence when *gyrA* of different organisms were aligned. The *gyrA* gene of various bacteria taxa including *Escherichi, Staphylococcus, Streptococcus, Mycobacterium,* etc were used (Figure 15). Conserved regions in organisms play important roles in basic cellular functionality, stability and reproduction. Modifications in such sequences are the foremost contributors to structural modifications and evolutionary relationships. Structures of the mutants A97V and H189Y together with the wild type were predicted and compared (Ramachandran plots yielded Z-score values for the Wild type to be 1.65 ± 0.27, A97V= 1.46 ± 0.27, and H189Y = 1.71 ± 0.27). Observation of predicted secondary structure shows the formation of a helix in A97V (in red rectangle) and the shortening of a helix in H189Y (in black rectangle) compared to the wild type (Figure 16). Tertiary structure comparison (of the cartoon and surface structure) of each mutant to the wild type shows slight conformational changes to the protein as a result of the variants (shown with arrows and rectangles in Figure 16 C – E). These structural changes may be giving rise to reduction in the binding affinity of levofloxacin or ciprofloxacin to the gyrase enzyme or topoisomerase (II) preventing the drug from efficiently performing the required function. In other studies on *gyrA* gene, mutations at A97V was determined to indicate resistance in levofloxacin (Malfertheiner et al., 2017) while Miftahussurur et al. (2019) recoded resistance

due to mutations in *gyrA* at the positions A197F, I194F, E193D. Other positions identified for *gyrA* mutations for levofloxacin resistance include mutations inside/outside the quinolone resistance-determining region (QRDR) such as between A71 to Q110 including A199V/I, R103H, H57Y, S63P, A88N/P/V, V65I, V77A, S83A, D86N, R130K, N87A/K/I/Y/ T, D91G/N/A/H/Y, D161N, A92T, D99V, A129T, D155N, V172I, P188S, D192N,) (Tshibangu-Kabamba & Yamaoka, 2021).

RdxA share similarity with other classical nitroreductases at a low percent identity of around 30 % and similarity of about 50 % (Goodwin et al., 1998). Therefore, it is a challenge to align nitroreductases from different organisms unlike *gyrA* enzyme. The predicted effect of amino substitution with respect to the region of the mutation could therefore not be predicted for mutations involving *RdxA* and *pbp*.

Metronidazole is a pro-drug that requires enzyme activation to perform its function. In *H. pylori* cells, this activation happens when metronidazole gets oxidized by ferredoxin or ferrodoxin via electrons donation from pyruvate oxidoreductase complexes. The activation results in the reduction of metronidazole nitro groups into forms capable of inhibiting nucleic acid synthesis (Arslan et al., 2017; Garrido-Treviño et al., 2022). Mutations in oxidoreductases such as *RdxA* gene (oxygen-insensitive NADPH nitroreductase) contributes to a decrease in action of nitroreductase enzymes.

Antibiotic resistance takes place when the rate of metronidazole activation inside the bacterium is negatively affected as a result of mutated *RdxA* gene (Garrido-Treviño et al., 2022; Megraud, 1997). Earlier studies have demonstrated association of metronidazole resistance to mutations in *RdxA* gene at positions R16C/H, Y103H, and S121D (Chang et al., 1997; King, 2021; Li et al., 2022). In the current study, all isolates proved phenotypically resistant to metronidazole. Analysis of *RdxA* gene sequences showed a number of amino acid substitutions (table 16). They included Q11Stop, R16H, E27G, I36T. Q50Stop, D61G and A67G. Other findings in this study were S70R, Y71F, E75K, V85G, R90G, G98S and H99P.

In an attempt to obtain information on what the amino substitutions could have on the structure of the protein, three (3) of the mutants, one from each of non-polar, polar charged and polar uncharged representative substituted amino acids were used for structure prediction. A few modifications were observed in the structures (Figures 23 - 25) when the cartoon and surface structures of mutants and wild type were compared (merged). It is suspected that these modifications may promote de novo inactivation of the *RdxA* gene leading to a damage to the helical structure of DNA by reducing hydroxylamine (Goodwin et al., 1998). Once these enzymes are deactivated through these conformational changes, they are unable to encode the enzymes required to reduce the prodrug metronidazole nitro groups into their bactericidal agent and as a result of an early truncation of activity (Goodwin et al., 1998; King, 2021; Tanih et al., 2010).

A recent study by King (2021) found two missense mutations in the *RdxA* gene at R16H and A118S/T. He found an isolate to contain both substitution while R16H was identified in one isolate just as is the case in the present study. Ferredoxin-like protein (fdxB) and NADPH flavin oxidoreductase (FrxA) are other nitroreductases that may be involved in low rate metronidazole resistance in *H. pylori* needing further investigation (King, 2021; Tanih et al., 2010).

Amoxicillin works by blocking the formation of bacterial cell walls when it interacts with penicillin binding proteins (PBPs). Point mutations in the transpeptidase region of the *pbp1A* gene, which codes for these PBPs, are typically the cause of amoxicillin resistance (Arslan et al., 2017; Garrido-Treviño et al., 2022; Saracino et al., 2021). The substitution of Threonine for Methionine at position 438 in this study was observed to produce the most distinct modification in structure (indicated with a black rectangle in surface structure of Figure 21 C-E). The most common mutations identified in amoxicillin resistant strains are substitutions at positions N562Y and S414R in the transpeptidase region. This study discovered mutations in PBP-encoding gene in the genomes of isolated *H. pylori* strains. Overall, there were 13 identified variants, three (3) of which were non-synonymous mutations. Mutations found included T254I, K315E and T438M all of which showed modified secondary structures when compared to the structure of the wild type (indicated with black rectangles) (Figure 19 - 21).. The most pronounced secondary structure modification was also seen in T438M and included deletion,

elongation and shortening of certain helices as well as elongation of strands at some positions (Figure 21 A & B).

All variants were related to amoxicillin-resistant phenotype. The mutations identified resulted in a conformational change in tertiary structures when they were individually aligned with the wild type. In T254I mutant, arrow-type helices (identified by red rectangles in cartoon structure of figure 19) are absent in the wild type. Structural changes as a result of mutations in PBP proteins can affect the inhibitory potential of amoxicillin on *H. pylori* cell walls synthesis via difficulty in the binding action of the drug to the organism. A compilation of common mutations in PBPs from other research findings include the following changes; D535N, F366L, V45I, S338R, V374L, S402G, N404S, S405N, S414R, L423F, S455N, N562(D/H/Y), N562(D/H), N562Y, N504D, S543 (H/R), T556S, T558S, A599(T/P/V), G595(del/A/S), T593(A/G/K/P/S), and Y637Ter (Tshibangu-Kabamba & Yamaoka, 2021).

Summary

The study looked at investigating the dynamics of prevalence of *H. pylori* in the Central Region of Ghana. To obtain a good representation of the population, secondary data which gave a larger sample size was utilised. *H. pylori* positive records were analysed for relationship with gender and year group as well as association with disease outcome. Gastric biopsies were obtained from study participants and processed for *H. pylori* related characteristics. *H. pylori* isolates were taken through antibiotic susceptibility testing against amoxicillin, clarithromycin, levofloxacin, metronidazole, amoxicillin-clavulanic acid, and

tetracycline. The investigation looked at the mutations in the genes causing antibiotic resistance. Secondary and tertiary structure conformational changes as a result of amino acid substitutions were elucidated to determine if such modifications exist and could impact of antibiotic activity. Thus, mechanism of action of the antibiotic resistance were determined by analysis of sequenced antibiotic resistant genes. The study also utilised questionnaires to obtain data on risk factors pertaining to infection in the region.

The prevalence of the disease was 77.09 % for males and 74.54 % for females with a general rate of 75.61 %. From 2012 to 2019, there was a general increase in prevalence with a slight reduction in rate in the year 2017 (57 – 67 %). The prevalence was high (at least 72.34 %) for all age groups. The maximum were obtained for 21- 30 and 31 – 40 at 77 % each. The highest disease outcome was observed for gastritis followed by esophagitis and then hiatal hernia. The relationships were found to be statistically significant for all three clinical conditions. Other conditions that showed significant relationship with infection were gastric ulcer, duodenal ulcer and portal hypertensive gastropathy. The highest percentage cases of ulcer and cancer were observed for older ages (over 40 years). Gastric cancer cases were more in males at 2.80 % compared to females with 0.39 %. All oesophageal cancer cases were seen in males. The disease conditions of *H. pylori* can therefore be said to be more pronounced in males than females.

A lot of factors are noted to influence *H. pylori* infection rates including marital status, level of education, level of income, lifestyle habits, etc. In the present

study, educational level, marital status, ethnic group, and job status were all identified to play a significant role in infection showing a statistically significant association with infection. Spicy food intake, source of drinking, personal and family history of dyspepsia, and duration of indication for endoscopy were all identified to be statistically significant to *H. pylori* infection. All other factors had no relationship with infection.

The most prevalent virulence factors were *dupA* (32.04 %) and *iceA2* (28.16 %) genes. While *dupA* showed no significant relationship with disease outcome, *iceA*2 was found to have a significant association with gastritis (p = 0.0005, OR = 7.78; CI = 2.27 to 25.70) and duodenal ulcer (p = 0. 0214, OR = 11.7; CI = 1.74 to 144). The *vacA* subtype with *m1* mid region showed a significant relationship with GU, DU, and varices. All other virulence factors identified in the bacterium had no significant relationship with infection.

Analysis of antibiotic resistant genes by the detection of *rdxA* gene for metronidazole resistance, *gyrA* for fluoroquinone (ciprofloxacin, levofloxacin) resistance and *pbp1* for amoxicillin resistant were performed on all DNA samples extracted from gastric biopsies. Amplification of these genes showed the percentage occurrence of these genes at 67.96 %, 45.63 %, and 35. 92 % for *gyrA*, *rdxA* and *pbp1* genes respectively. Based on the detection of more than one of these genes in individual samples, it can be said that there are several cases of multiple drug resistance in some participants. Triple resistance to antibiotics was observed at 16.50 % after detecting 17 out of 103 positive samples to contain the combination of *rdxA*, *gyrA*, and *pbp1* resistant genes.

Phenotypic detection of resistance showed complete (100 %) resistance to metronidazole, amoxicillin-clavulanic acid, and clarithromycin. A resistant rate of 20 % was determined for tetracycline and ciprofloxacin while levofloxacin recorded 40 % resistance.

Mutations exist in the *RdxA*, *gyrA*, and *pbp* genes of *H. pylori* and are linked to antibiotic resistance. Amino acid substitutions are capable of causing structural modifications to protein structures which can interfere in the activity of antibiotics against the target organism,

Conclusions

Though not significant, the prevalence of *H. pylori* infection is higher in males (77.09 %) than females (74.54 %) and the trend in prevalence from 2012 to 2019 has been high with at least 60 % rate. The presence of *H. pylori* was found to be significantly associated with a number of diseases including esophagitis, duodenal ulcers, hiatal hernias, and portal hypertensive gastropathy.

Risk factors such as level of education, marital status, job status, duration of indication for endoscopy, presence of personal or family history of dyspepsia, source of drinking water, and preference for spicy food are significant in association with *H. pylori* infection.

The main *H. pylori* virulent factors that were identified in the samples analysed were *iceA2*, *cagE,* and *dupA.* While *iceA2* and *vacA* m1 subtype had significant association with some disease outcomes, other factors identified showed no link to disease.

The resistance rate of metronidazole, amoxicillin, amoxicillin-clavulanic acid, and clarithromycin to *H. pylori* is high within the study area. Ciprofloxacin, levofloxacin, and tetracycline are better options owing to relatively improved susceptibility results in the study. Again, there are mutations in metronidazole, levofloxacin and amoxicillin target genes in *H. pylori* which may be implicated in drug resistance. The resultant amino acid substitutions may result in structural modifications of the protein.

Recommendation

Based on the findings from this study it is recommended that:

1. The current treatment guideline be looked at again and possibly replaced or modified
2. Further studies should consider a larger sample size to obtain larger data for antibiotic resistance which will consider other antibiotics such as gentamycin, doxycycline, moxifloxacin, rifabuten, etc.
3. Other virulent factors such as *OipA*, *BabA*, and a repeat of the *vacA* subtypes needs further investigation.
4. Different mutations were identified to be associated with antibiotic resistance. However, there may be a possible skewed analysis owning to the limited number of isolated strains and hence a challenge to generalizing findings to a larger population in Ghana. A larger number of isolates will create a better understanding of the development and level of antibiotic resistance in *H. pylori* in Ghana.

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APPENDICES

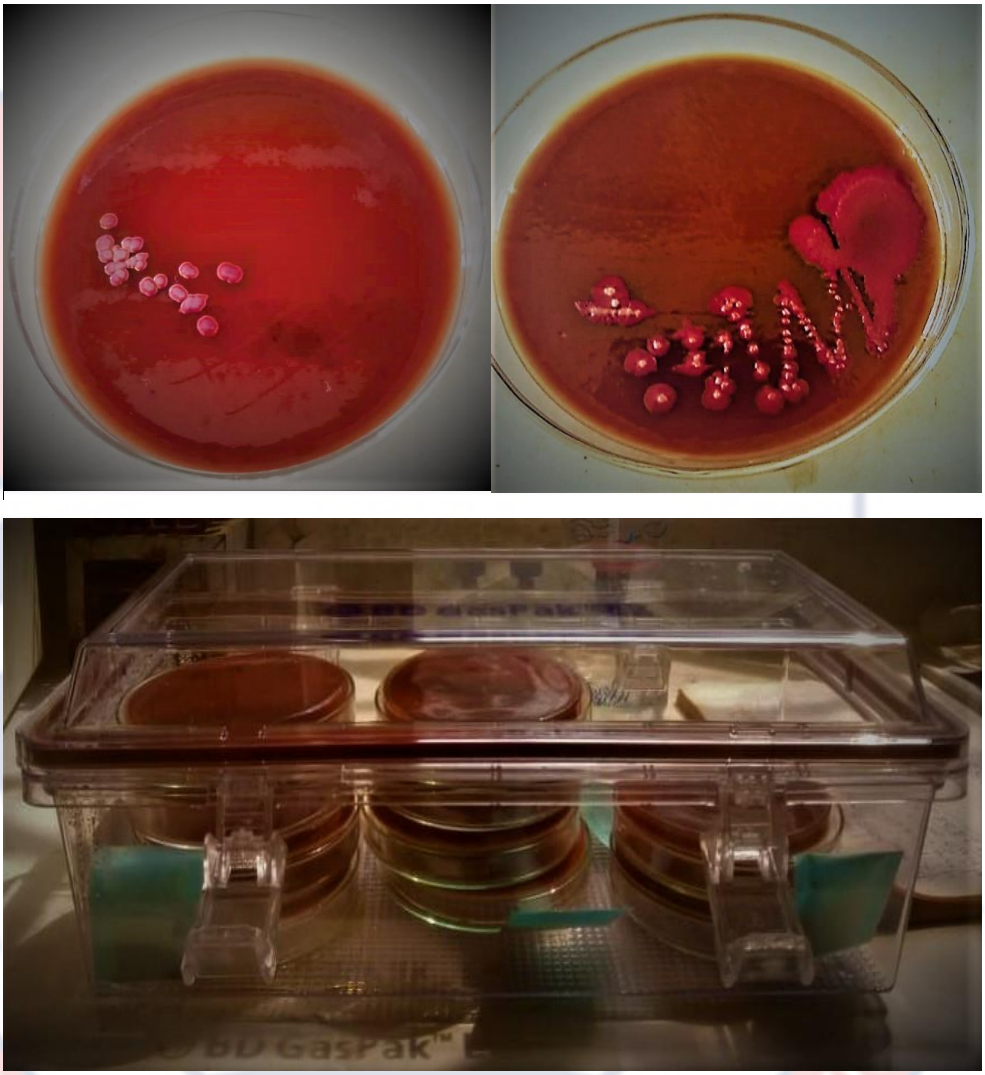
Appendix A: Frequencies of antibiotic resistant genes in various gastro-duodenal pathologies

Gene combination	Gast. n=65	GU n=14	DU	DN	GC	HH	GERD	varices
rdxA	25	3	2	1	3	3	3	3
gyrA	27	9	2	3	2	9	14	2
pbp1	17	5	2	0	0	2	5	3
rdxA+pbp1	3	0	0	0	0	0	0	2
rdxA+gyrA	7	0	0	1	2	3	1	1
pbp1+gyrA	1	2	0	0	0	2	2	1
rdxA+pbp1+gyrA	10	3	2	0	0	0	2	0
rdxA only	5	0	0	0	1	0	0	0
pbp1 only	3	0	0	0	0	0	1	0
gyrA only	9	4	0	2	0	4	9	0
none	27	5	0	0	0	9	8	0

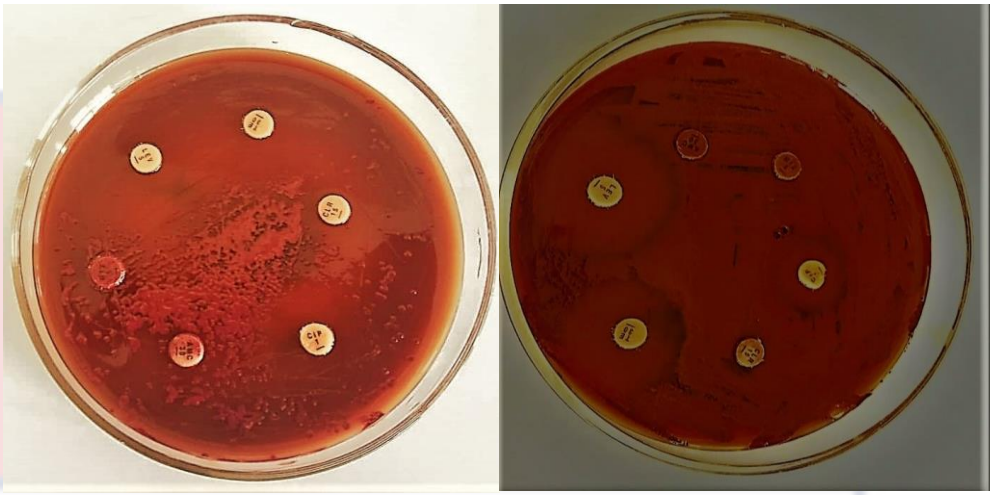
Appendix B: Measured zones of inhibition for various antibiotics in the culture of gastric biopsy samples for *H. pylori* culture

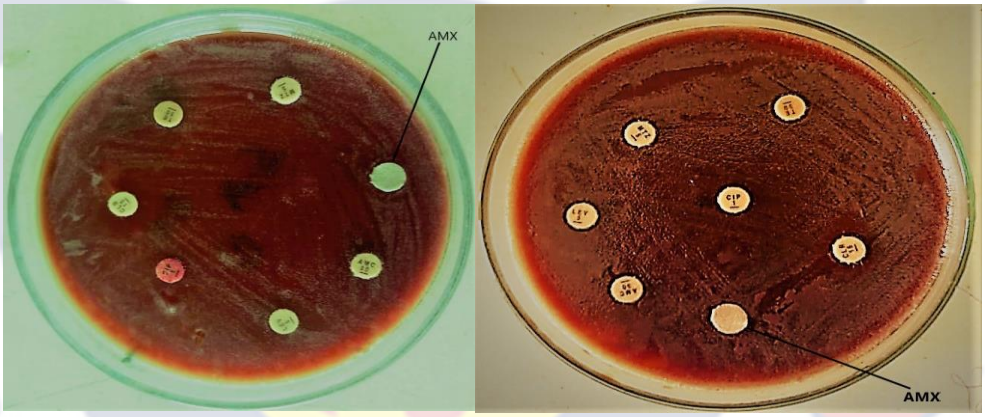
Sample ID	Zones diameter (cm) measured for various antibiotics						
	LEV	TET	CIP	MTZ	CLR	AMC	AMX
1	2.6	2.2	2	0	0	0	0
2	2.6	2.2	2.2	0	0	0	0
3	1.1	0.7	1.2	0	0.6	0	0
4	1.2	0.8	1.1	0	0	0	0
5	0	0	0	0	0	0	0
6	3.4	2.8	2.2	0	0	0	0
7	3.5	2.6	2	0	0	0	0
8	3.1	2.8	2.1	0	0	0	0
9	3.2	2.7	2.3	0	0	0	0
10	3	2.7	1.9	0	0	0	0
11	3.1	2.7	2	0	0	0	0
12	2.9	2	1.9	0	0	0	0
13	2.6	2.3	2	0	0	0	0
14	2.9	2.4	1.8	0	0	0	0
15	2.9	2.6	1.8	0	0	0	0

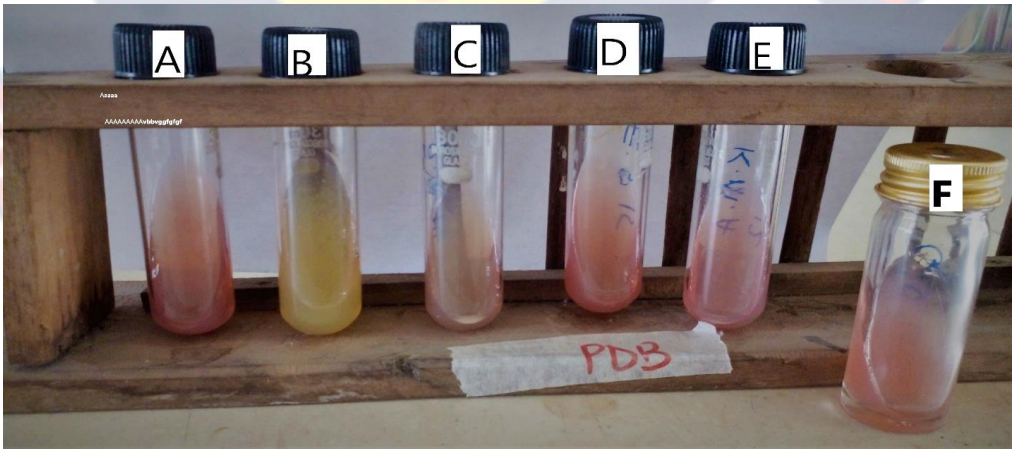
Appendix C: Culture plates showing the growth of *H. pylori* after direct culture of gastric biopsies



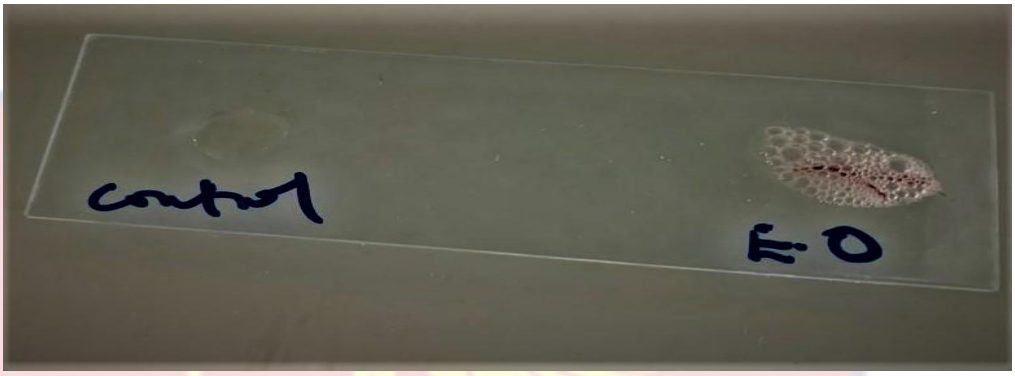
Appendix D: Antibiotic susceptibility plates and catalase test for the presence of *H. pylori*







Appendix E: Catalase and Oxidase results and a view of gram stained *H. pylori*

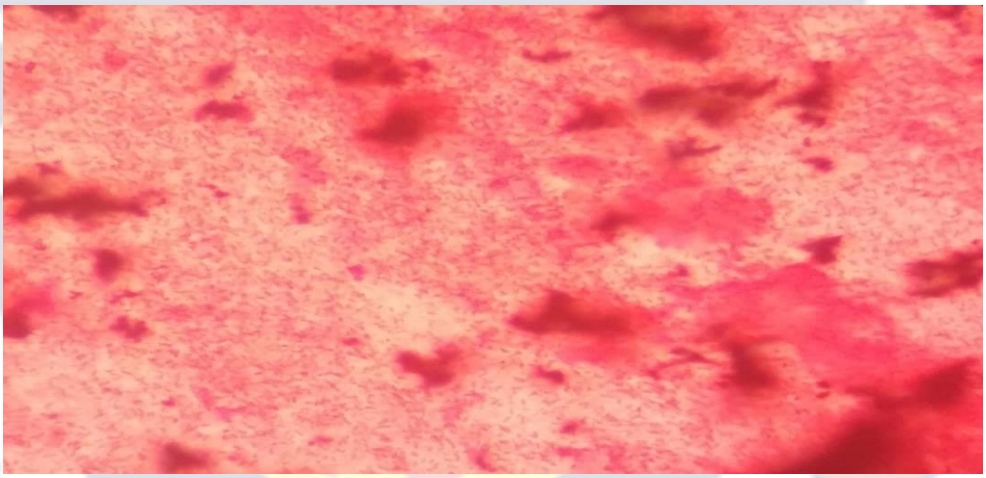


Catalase test results for E.O

Oxidase test results showing positive results for sample G.O and negative for the control.

Oxidase test results showing positive results for sample K.O and negative for the control.

Rehydration of extracted DNA samples



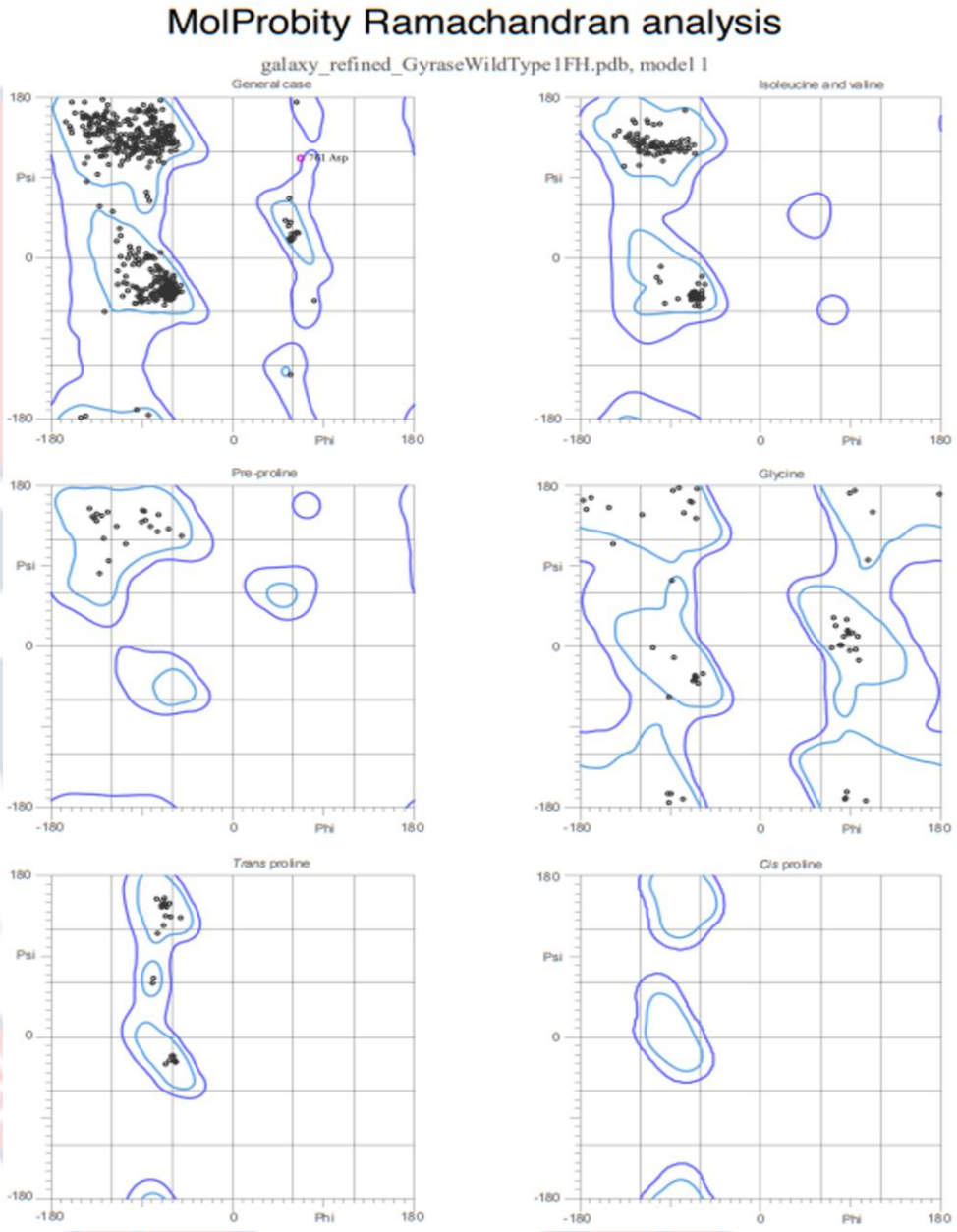
Microscopic view of gram negative rods after gram staining to confirm the presence of *H. pylori*

Appendix F: Sample online data for determination of mutated nucleotide position

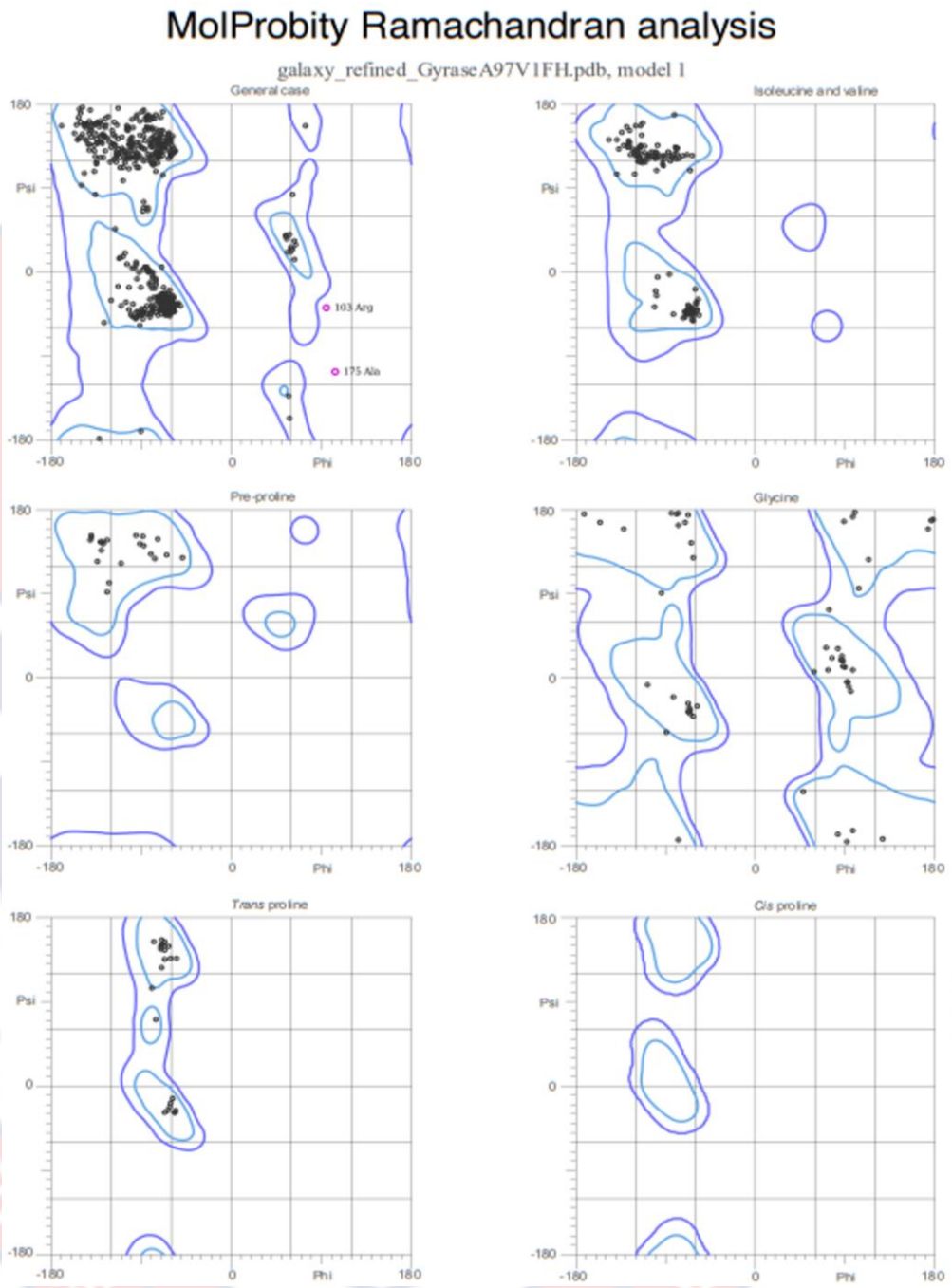
```
>chromosome:ASM852v1:Chromosome:1013763:1014384:-1
1014384 GGTTGCTGATTGTGGTTTATGGTTTGGGGTATTCCTTGATGCGCTTTATTGCGGAATTTT 1014325
1014324 ACAGAGAGCCGGACAGCCAAATGGGGGTTTATTTTTTAAATTTGAGCATGGGGCAGATTT 1014265
1014264 TAAGCTTATTTATGGTAATTGTTTCGTTAGGGATTTTATTGTATGCTACAAAAAATTCTA 1014205
1014204 AAAAAATAAAGGAAAATCAATGAAATTTTTGGATCAAGAAAAAAGAAGACAATTATTAAA 1014145
1014144 CGAGCGCCATTCTTGCAAGATGTTTGATAGCCATTATGAGTTTTCTAGCACAGAATTAGA 1014085
1014084 AGAAATCGCTGAAATCGCCAGGCTATCGCCAAGCTCTTACAACACGCAGCCATGGCATTT 1014025
1014024 TGTGATGGTTACTGATAAGGATTTAAAAAAACAAATTGCAGCGCACAGCTATTTCAATGA 1013965
1013964 AGAGATGATTAAAAGCGCTTCAGCGTTAATGGTGGTATGCTCTTTAAGACCCAGCGAGTT 1013905
1013904 GTTACCACACGGCCACTACATGCAAAATCTCTATCCGGAGTCTTATAAAGTTAGAGTGAT 1013845
1013844 CCCCTCTTTTGCTCAAATGCTTGGCGTGAGATTCAACCACAGCATGCAAAGATTAGAAAG 1013785
1013784 CTATATTTTAGAGCAATGCTAT                                       1013763

>chromosome:ASM878v1:Chromosome:591883:592503:-1
592503 GATAGTTACAGAAACGAGCACCGGTAAGATTTTAGCCTTAGTGGGGGGGATTGATTATAA 592444
592443 AAAAAGCGCTTTCAATCGCGCCACGCAAGCCAAACGGCAGTTTGGGAGCGCGATCAAGCC 592384
592383 TTTTGTGTATCAAATCGCTTTTGATAATGGCTATTCCACCACTTCCAAAATCCCTGATAC 592324
592323 CGCGCGAAATTTTGAAAATGGCAATTATAGTAAAAACAGCGTGCAAAACCACGCATGGCA 592264
592263 CCCTAGCAATTATACTCGCAAATTTTTAGGGCTTGTAACCTTGCAAGAAGCCTTGAGCCA 592204
592203 TTCGTTAAATCTGGCTACGATTAATTTAAGCGATCAGCTTGGCTTTGAAAAAATTTATCA 592144
592143 ATCTTTAAGCGACATGGGGTTTAAAAACCTCCCTAAAGATTTGTCTATTGTGTTAGGGAG 592084
592083 CTTTGCTATCTCACCGATTGATGCGGCTGAAAAGTATTCTCTATTTTCTAATTACGGCAC 592024
592023 CATGCTCAAACCCATGCTCATTGAAAGCATCACCAACCAACAAAACGAAGTCAAAACTTT 591964
591963 CACGCCCATTGAAACCAAAAAGATCACTTCCAAAGAACAAGCTTTTTTAACCCTTTCAGC 591904
591903 GCTGATGGATGCGGTAGAAAA                                       591883

>chromosome:ASM878v1:Chromosome:714743:715364:1
714743 TTATTTTAATTAAATTTTAGGGAGACACATGCAAGATCATTTAGTCAATGAAACAAAAAA 714802
714803 TATTGTAGAAGTGGGGATTGATTCTTCTATTGAAGAGAGCTATTTGGCTTATTCCATGAG 714862
714863 CGTGATCATAGGGCGCGCTTTACCGGACGCTAGAGACGGCTTAAAGCCTGTGCATAGGCG 714922
714923 TATTTTGTATGCGATGCATGAATTAGGCCTTACTTCCAAAGTCGCTTATAAAAAAAGCGC 714982
714983 TAGGATCGTGGGTGATGTGATTGGTAAATACCACCCCCATGGCGACACCGCAGTTTATGA 715042
715043 TGCGTTAGTGAGAATGGCGCAAGATTTTTCTATGCGCTTGGAATTAGTGGATGGGCAGGG 715102
715103 CAACTTTGGCTCTATTGATGGCGATAACGCCGCAGCGATGCGTTACACTGAAGCCAGAAT 715162
715163 GACCAAGGCGAGTGAAGAGATTTTAAGAGATATTGATAAAGACACCATTGATTTTGTGCC 715222
715223 TAATTACGATGACACCTTAAAAGAGCCAGATATTTTACCAAGCCGTCTGCCTAACCTTTT 715282
715283 AGTCAATGGGGCTAATGGGATCGCCGTAGGGATGGCGACTTCTATCCCCCCTCATAGGAT 715342
715343 TGATGAAATCATAGACGCTTTA                                       715364
```

Sample sequences from ensemblbacteria database for the determination of position of nucleotides involved in mutations in various genes.

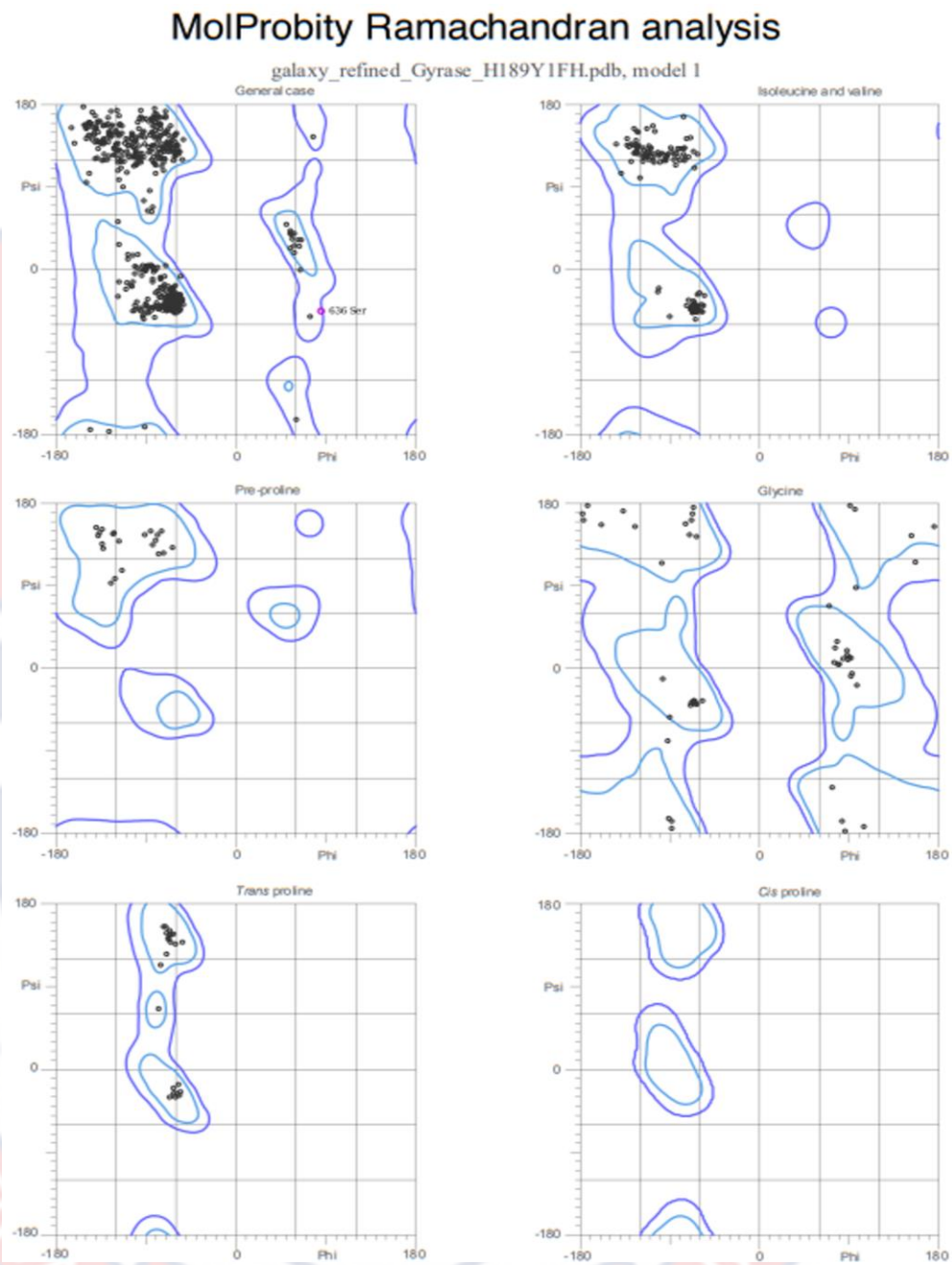
Appendix G: Sample Ramachandran plots for predicted structures of wild type and mutant genes



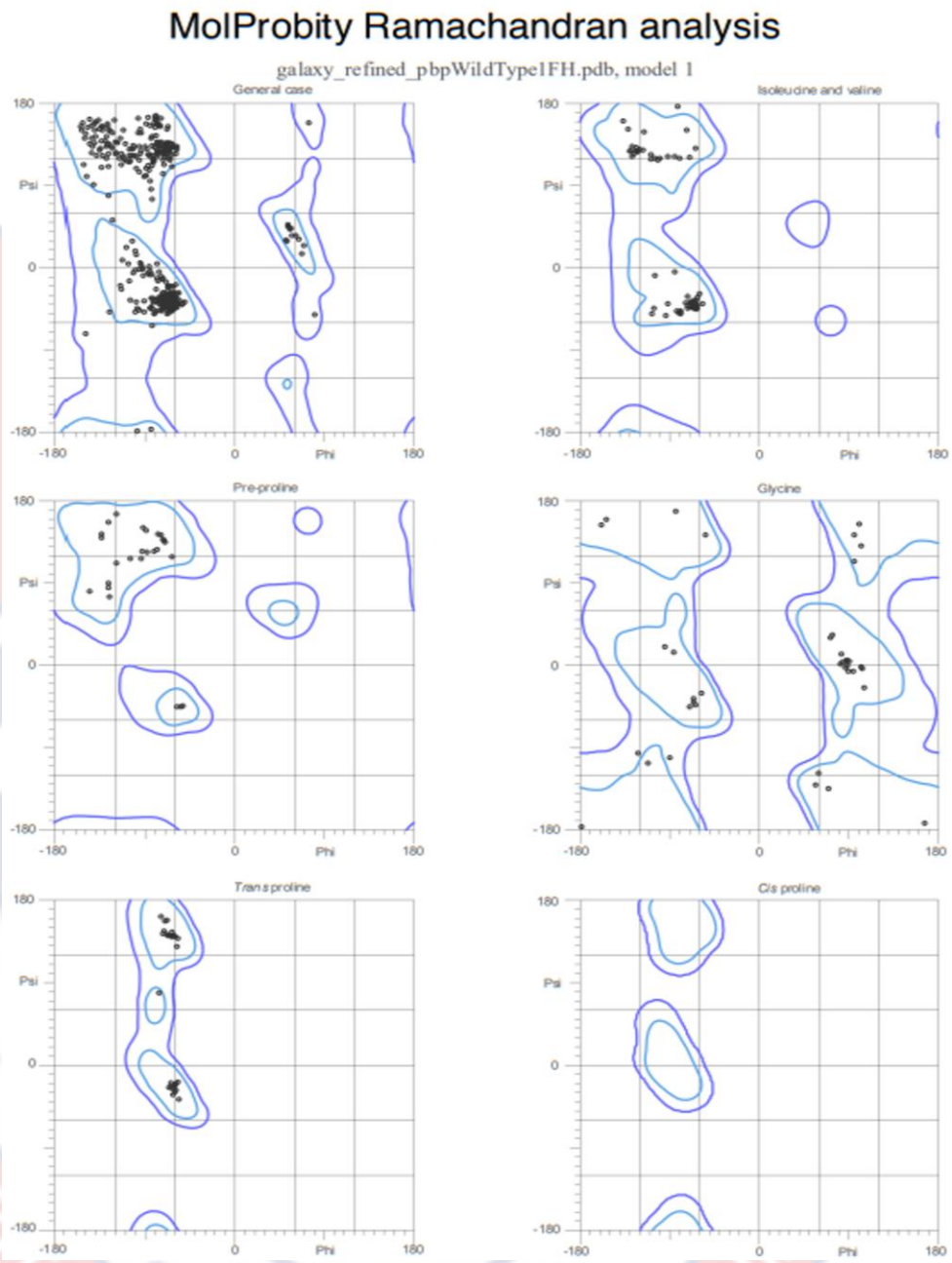
Ramachandran plot for *gyrA* wild type analysis showing that 98.5% (813/825) of all residues were in favoured (98%) regions. 99.9% (824/825) of all residues were in allowed (>99.8%) regions. There was 1 outliers (phi, psi): 761 Asp (68.8, 113.7)



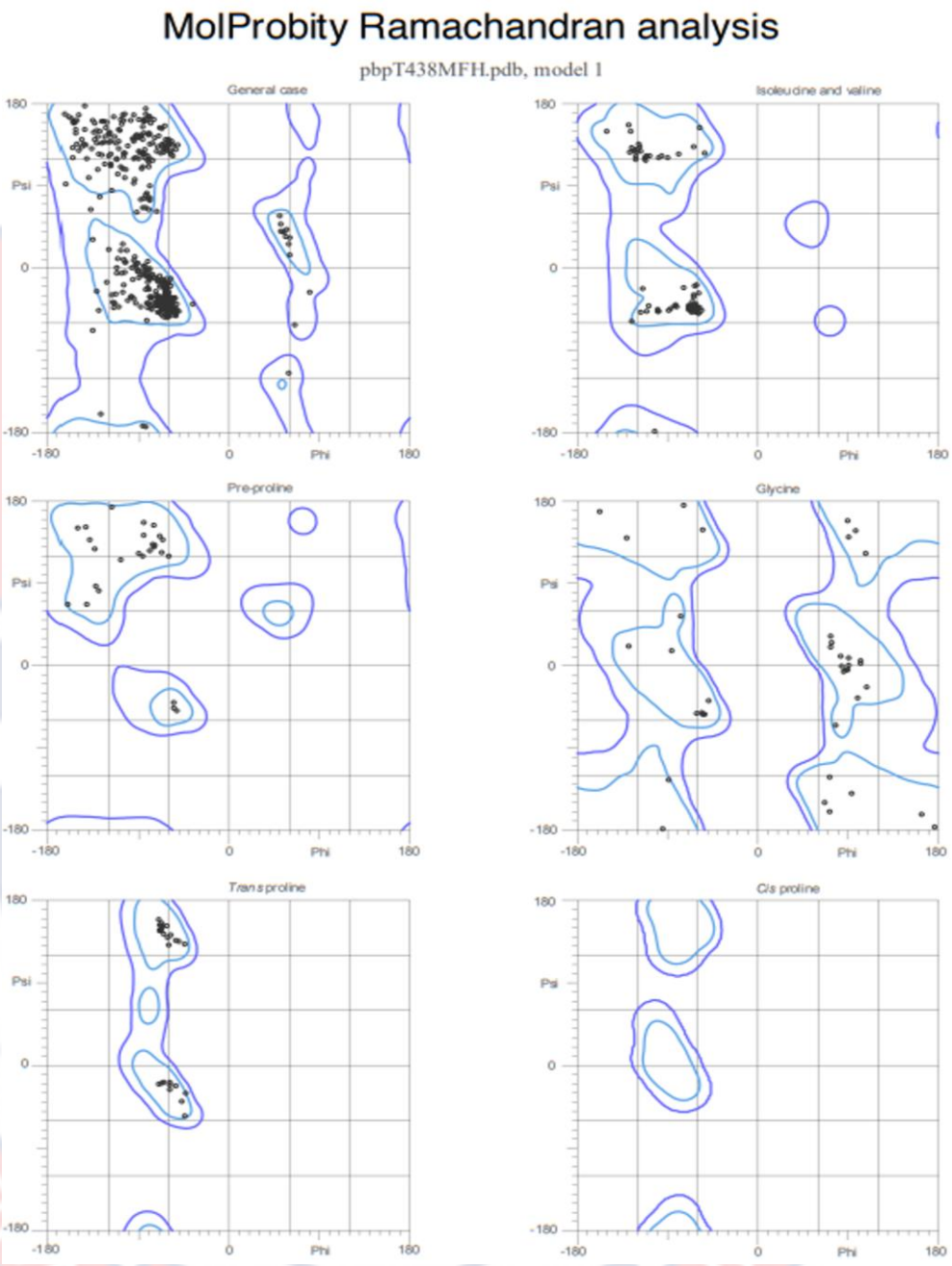
Ramachandran plot for *gyrA* A97V mutant showing that 97.9% (808/825) of all residues were in favoured (98%) regions and 99.8% (823/825) of all residues were in allowed (>99.8%) regions. There were 2 outliers (phi, psi): 103 Arg (96.4, -39.0) 175 Ala (105.2, -107.2).



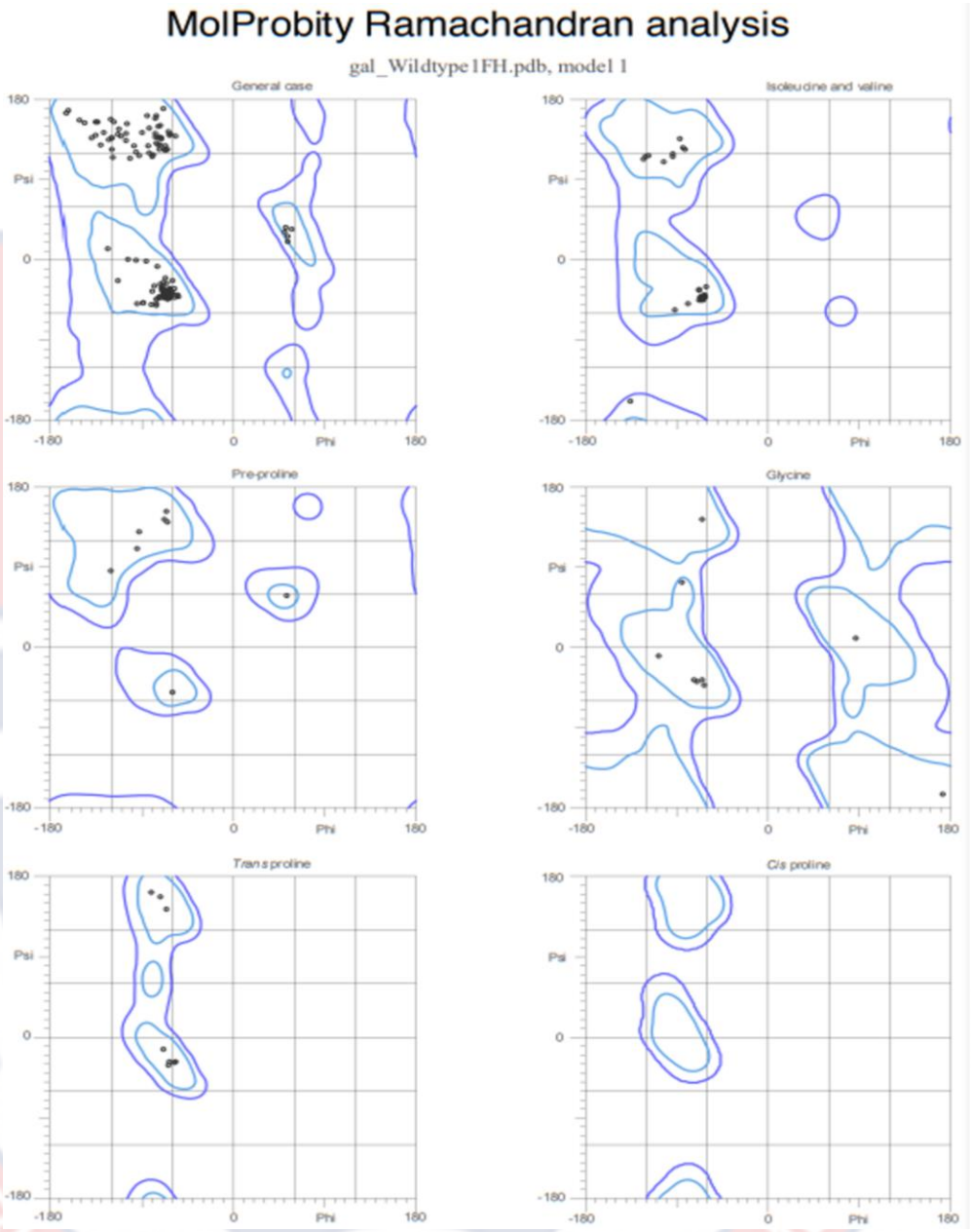
Ranachandran plot for *gyrA* H189Y showing that 98.4% (812/825) of all residues were in favoured (98%) regions. 99.9% (824/825) of all residues were in allowed (>99.8%) regions. There were 1 outliers (phi, psi): 636 Ser (86.4, -45.5)



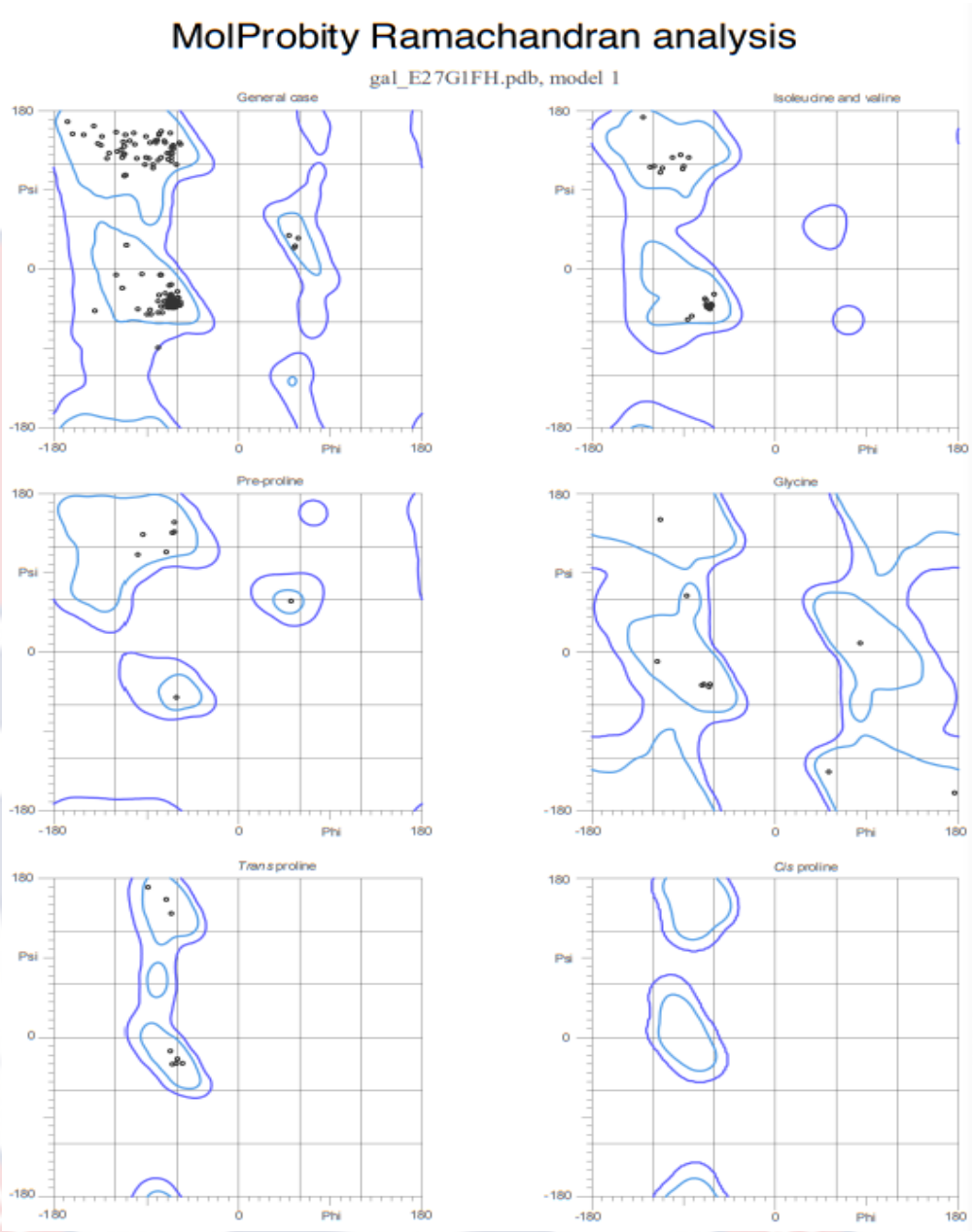
Ramachandran plot for *pbp* wild type showing that 98.5% (647/657) of all residues were in favoured (98%) regions. 100.0% (657/657) of all residues were in allowed (>99.8%) regions. There were no outliers



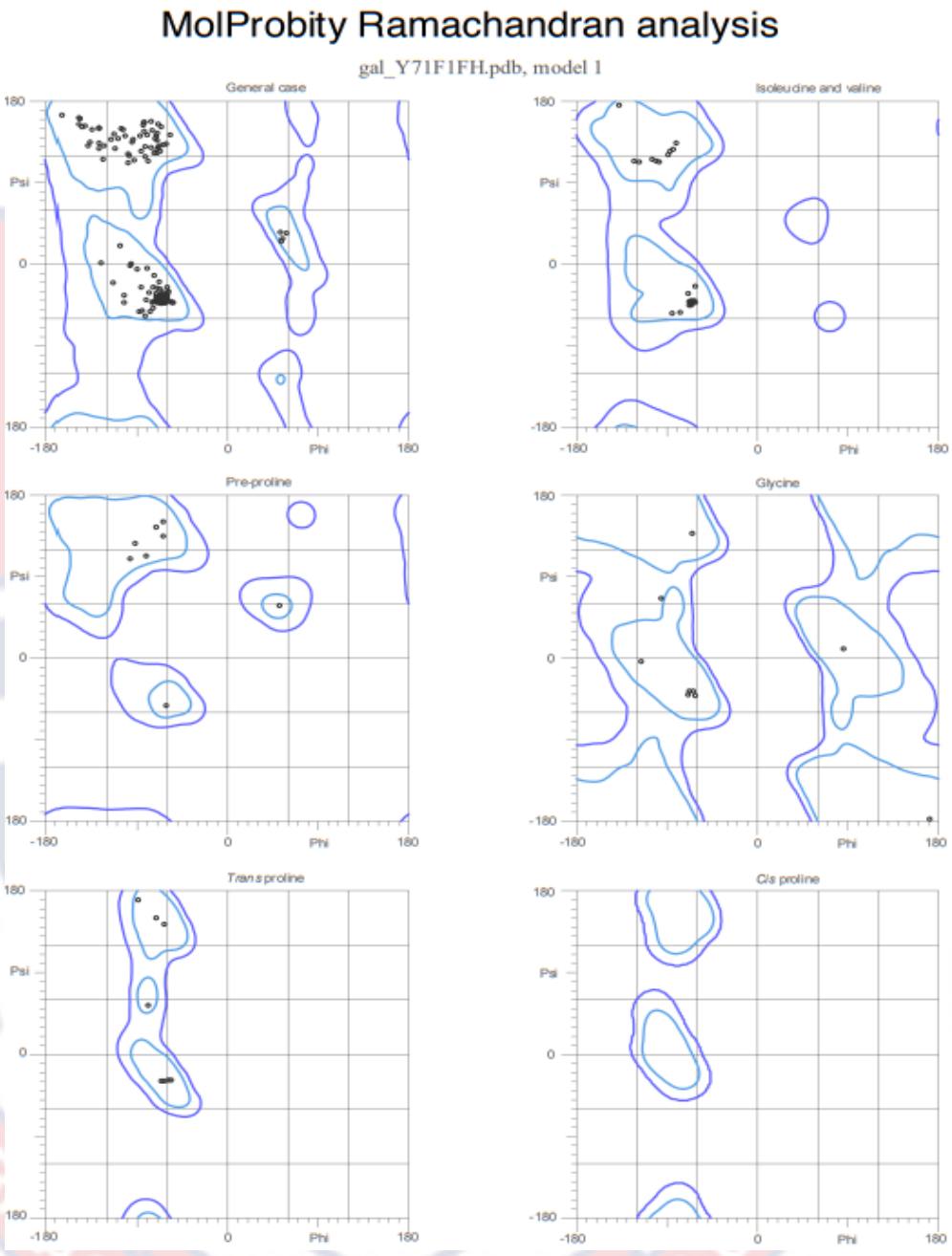
Ramachandran plot for *pbp* T438M showing that 97.7% (642/657) of all residues were in favoured (98%) regions. 100.0% (657/657) of all residues were in allowed (>99.8%) regions. There were no outliers.



Ramachandran plot for *RdxA* wild type showing that 99.5% (207/208) of all residues were in favoured (98%) regions. 100.0% (208/208) of all residues were in allowed (>99.8%) regions. There were no outliers.



Ramachandran plot for *RdxA* E27G mutant showing 99.0% (206/208) of all residues were in favoured (98%) regions. 100.0% (208/208) of all residues were in allowed (>99.8%) regions. There were no outliers.



Ramachandran plot for *RdxA* Y71F mutant showing 99.5% (207/208) of all residues were in favoured (98%) regions. 100.0% (208/208) of all residues were in allowed (>99.8%) regions. There were no outliers