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Mutational analysis of antibiotic resistance genes in *Helicobacter pylori* from Ghanaian dyspepsia patients: Implications for treatment strategies

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ABSTRACT

Background: Antibiotic resistance jeopardizes the effectiveness of conventional treatment regimens for *Helicobacter pylori* infections, and this remains a major global health concern. *H. pylori* genes mutations negatively affect actions of most first line antibiotics. This study aimed to perform mutational analysis on *H. pylori* antibiotic resistance genes in Ghanaian patients diagnosed with dyspepsia.

Materials and methods: Antrum gastric biopsies were taken from 169 study participants, minced in Brain Heart Infusion broth and cultured. Sensitivity to antibiotics of *H. pylori* isolates was determined by disc diffusion. Extracted DNA were amplified and antibiotic resistance genes *gyrA*, *pbp1*, and *rdxA* sequenced. Resistance genes were analysed for base and point mutations using online databases and Ugene 45.0 software.

Results: Using rapid urease test, *H. pylori* infection prevalence was estimated to be 61%. Phenotypically, no sensitivity was recorded for metronidazole, amoxicillin, clarithromycin, and amoxicillin-clavulanic acid against the tested isolates. Resistance to levofloxacin was found to be 40% while 20% was recorded for each of tetracycline and ciprofloxacin. Mutations identified included G242 C/A, T254I, and S417T for *pbp1* gene in amoxicillin resistance; K2N, Q6H, Q50Stop, E75K, R90K, G98S, H99P, R131K, and A183V for *rdxA* gene; N87I/T, A97V, M191I, V199 M/A, H200Y, and G208E for *gyrA* gene in levofloxacin resistance.

Conclusions: There is high *H. pylori* antibiotic resistance in the region with amoxicillin, metronidazole, amoxicillin-clavulanic acid and clarithromycin showing no sensitivity to tested isolates. Tetracycline and ciprofloxacin may be more appropriate therapeutic regimen options against *H. pylori*. Observed resistance could be due to mutations in *rdxA*, *pbp1,* and *gyrA* genes.

1. Introduction

Helicobacter pylori (*H. pylori*) which is known to inhabit the human stomach has been linked to a number of gastrointestinal conditions, including gastritis, peptic ulcers, and gastric cancer.(Sjomina et al., 2018).The bacterium is thought to be a chronic infectious pathogen that affects over 50% of the population worldwide (Kuna et al., 2019). All human populations are at a risk of this infection, but those from poorer socioeconomic backgrounds and those who live in unhygienic, crowded housing are more vulnerable (Yuan et al., 2022). Because of this, it may be said that infection is nearly universal in developing nations, with a prevalence of roughly 90% as opposed to 25% in developed countries

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(Hooi et al., 2017; Zamani et al., 2018). Many infected individuals do not exhibit any symptoms at all. Nonetheless, about 20% of those infected with this bacterium experience severe illnesses such as lymphomas and peptic ulcers (Malfertheiner et al., 2023). In an attempt to optimize treatment efficacy and avoid the development of resistance, distinctive therapeutic approaches are used to treat *H. pylori* infection. A triple medication combination comprising a proton pump inhibitor, metronidazole, and an additional antibiotic (either amoxicillin or clarithromycin) is used as the first line (Nishizawa et al., 2009), with tetracycline being substituted for those with penicillin allergies. In instances where there is treatment failure with first line triple therapy, levofloxacin based triple therapy or quadruple therapy becomes an option (Chey et al., 2018). A number of studies have reported increasing antibiotic resistance originating from point mutations in antibiotic related genes in many places around the world, which may explain why treatment failure is also increasing.(Hasanuzzaman et al., 2024; Yu et al., 2024). Geographic variations are also observed in the occurrence of antibiotic resistance resulting partly from the general population's extensive, unchecked, and inappropriate usage of antibiotics. This includes the inappropriate use of antibiotics for viral infections, non-infectious fevers, respiratory and gastrointestinal diseases as well as self-medication, incomplete dosing and sharing of medicines(Ferrara et al., 2024; Tangcharoensathien et al., 2018).

Genetic determinants such as *gyrA*, *rdxA*, and *pbp1A* genes are prominent and intricately intertwined into the resistance mechanisms of *H. pylori* (Fauzia et al., 2023). DNA gyrase protein is encoded by *gyrA* gene, an essential part of DNA repair and replication. The structural integrity of bacterial DNA is preserved during replication by the action of DNA gyrase which is a viable target by fluoroquinolone antibacterial agents like levofloxacin, ciprofloxacin, moxifloxacin, etc. These drugs inhibit this enzyme resulting in impaired DNA replication. Mutations in *gyrA* results in modification of the antibiotic's binding affinity to its target site compromising the effectiveness of the agent(Fauzia et al., 2023). Similarly, resistance to nitroimidazole antibiotics like metronidazole is linked to the *rdxA* gene, which codes for an essential part of the bacterial respiratory chain. The first-line treatment of *H. pylori* infections is largely dependent on nitroimidazoles(Wilcox, 2017), and figuring out the genetic variants in *rdxA* holds the potential to understand the complex processes that contribute to antibiotic resistance. Another commonly used component of *H. pylori* antibiotic medication is penicillin-based medicines (beta-lactam antibiotics) like amoxicillin, cloxacillin, and ampicillin whose mode of action is related to inhibiting effective bacterial cell wall production by interfering with penicillin binding proteins (PBPs). PBPs, encoded by *pbp1* gene, are responsible for the regulation of assembly of the peptidoglycan layer of bacterial cell walls (Bandara et al., 2009). In view of this, changes in *rdxA, pbp1*, and *gyrA* genes can result in resistance, reducing the effectiveness of these commonly used antimicrobials. The dearth of knowledge regarding the antibiogram and genes causing antibiotic resistance in *H. pylori* in Ghana may be impeding efforts to eradicate the infectious agent. Based on this, it is hypothesized that mutations in antibiotic resistance genes of *Helicobacter pylori* contribute to the antibiotic resistance observed in Ghanaian patients diagnosed with dyspepsia. This hypothesis is grounded on the findings that specific mutations in the rdxA, pbp1, and gyrA genes are associated with resistance to commonly used antibiotics, including amoxicillin, metronidazole. As antibiotic resistance continues to rise worldwide, understanding the molecular basis for resistance in *H. pylori* is crucial for developing effective treatment strategies. The identification of specific mutations can help clarify how these bacteria evade treatment. The main objective of this research was to perform mutational analysis on *H. pylori* antibiotic-resistance genes in Ghanaian patients diagnosed with dyspepsia, and explore the molecular mechanisms underlying *H. pylori* resistance specifically linked to *gyrA, rdxA, and pbp1* gene mutations.

2. Materials and methods

2.1. Ethical considerations and subject recruitment

Sample acquisition begun after approval of the study by review committee of the Ethical Review Board, University of Cape Coast, Ghana and given the clearance number UCCIRB/CANS/2019/05. The purpose and nature of investigation was disclosed to all participants who were referred for upper gastrointestinal endoscopy. The study was explained to participants in either English or the appropriate indigenous language for those who did not understand English. This took place following the routine nurse-led pre-procedure patient counselling and education session. A skilled gastroenterologist carried out each endoscopic procedure. Participants completed a standard questionnaire and additional endoscopic biopsies obtained after informed consent has been signed. No compensation was offered for participation in the study. Individuals who opted out of the study underwent the scheduled endoscopy procedure with no extra biopsies taken. All procedures were performed in compliance with relevant laws and institutional guidelines.

2.2. Study design and sites

A purposive sampling technique was used. Study was carried out at the Life Sciences Medical Centre and Oak Tree Medical Services esophagogastroduodenoscopy units. These health facilities serve the residents of Cape Coast and also receive requests for endoscopy services from medical facilities in other parts of Central Region, and occasionally Western Region of Ghana. All patients referred to the facilities and consented were enrolled onto the study. Collection of gastric biopsy samples at the endoscopy centers span between October 2019 and August 2021.

2.3. Inclusion criteria

All referred adult patients for esophagogastroduodenoscopy were taken into consideration for inclusion. Parents or older relatives who are over the age of 18 gave their approval on behalf of patients under the age of 18. This was because in the Ghanaian jurisdiction, individuals under 18 years are classified as minors and the laws of the country require that minors cannot provide legally binding consent on their own. Patients included were persons who had been sent to endoscopy referral centers because they had a history of gastritis or peptic ulcer disease (PUD). Also included were patients with persistent or recurring dyspepsia or epigastric discomfort, unexplained iron deficiency anemia, or idiopathic thrombocytopenic purpura.

2.4. Exclusion criteria

The investigation excluded individuals who were clinically unstable, those with a high risk of bleeding, or with substantial organ failure, such as dysfunction of the kidney, liver, or heart. Individuals who had a stricture or other obstruction that prevented complete access or clear endoscopic inspection, per the gastroenterologist's observations, were also disqualified.

2.5. Collection and transportation of gastric biopsies

The endoscopic procedure was a standard investigation to determine the underlying reason of the illnesses of patients who were present at the facility. Each patient (total = 169) had two additional antral stomach biopsies obtained once they consented to participate in the study. Gastric biopsies obtained from every patient were tested for the presence or absence of *H. pylori* using rapid urease tests (HelicotecUT®Plus, Strong Biotech Corp-Taiwan). Both *H. pylori*-positive and *H. pylori*-negative biopsies were subsequently transferred into brain Heart Infusion Broth (BHI) transport medium and transported on ice to the

laboratory for culture and DNA extraction.

2.6. Isolation and identification of H pylori from gastric biopsies

Aliquots of the transport medium were used to individually homogenize all biopsy specimens before they were streaked onto the modified BHI blood agar. The inoculated plates were incubated for a maximum of 10 days at 37 °C in a microaerophilic environment. Biochemical tests for urease, catalase, oxidase, and bacterial morphology were carried out in order to verify the presence of *H. pylori*. After single colonies were obtained through sub-culturing, the isolates were stored at −20 °C in sterile 20% glycerol-BHI broth.

2.7. Antibiotic susceptibility testing of H. pylori isolates

Antibiotic susceptibility test was carried out on the pure *H. pylori* isolates (N = 15) using metronidazole (5 μg), amoxicillin (10 μg), levofloxacin, clarithromycin (15 μg), amoxicillin-clavulanic acid (10 μg), tetracycline, and ciprofloxacin (5 μg), following a previously described procedure with slight modification (Gehlot et al., 2016). For each *H. pylori* isolate, a bacterial suspension was prepared and its turbidity was adjusted to the 0.5 McFarland standard. Standardized bacterial suspension aliquots were pipetted unto the surface of a sterile Brain Heart Infusion (BHI) agar plate containing 7% laked horse blood (Thermofischer Scientific, UK) and spread uniformly with a sterile glass spreader. The antibiotic discs were subsequently placed on the inoculated agar plates which were incubated overnight at 37 °C under microaerophilic conditions. Zone diameter breakpoints used in the testing of ciprofloxacin, metronidazole, tetracycline, and clarithromycin and levofloxacin effectiveness were 14 mm, 17 mm, 10 mm, and 16 mm and 29 mm, respectively.(Tang et al., 2020; Tanih et al., 2010).

2.8. Bacterial DNA extraction

DNA of bacteria was extracted from single colonies of sub-cultured isolates, using the Wizard Genomic DNA purification Kit (Promega Corporation, USA), following manufacturer's protocol. Concentration and purity of the DNA samples were determined using SimpliNano (Biochrom, USA). DNA samples were kept at −20 °C for further analyses.

2.9. Molecular characterization and screening for antibiotic resistance genes

The specific primer *Hp23Sr6/r7* was used for molecular detection of *H. pylori* using PCR under predetermined conditions. All primer sequences for molecular characterization and detection of antibiotic resistance genes are shown in Table 1 (Matteo et al., 2008; Pourakbari et al., 2018; Suzuki et al., 2013; Zhang et al., 2020). PCR was run using each primer set and OneTaq® 2X Master Mix with Standard Buffer following manufacturer's protocol with slight modification. Each cycle condition was set for 30 cycles, and PCR products separated on 2 % agarose gel stained with ethidium bromide.

The antibiotic resistance targeted sequences for *gyrA*, *rdxA* and *pbp1A* genes of the isolated *H. pylori* strains were sent for Sanger sequencing at Inqaba Biotec, West Africa. Analysis of DNA sequences and determination of similarities were done with UGENE 45.0 software and online BLASTX network available at the National Centre for Biotechnology Information website (NCBI) (http://www.ncbi.nlm.nih.gov). Alignment was done with MUSCLE using UGENE 45.0 software. Mutation in resistance genes were compared to *H. pylori* strain ATCC 26695 reference sequences. Nucleotide translation to amino acid sequences for analysis of mutational changes was done using https://web.expasy.org/translate/. Point mutation detections were based on presence of non-synonymous mutations in *pbp1* gene sequence for amoxicillin and amoxicillin-clavulanic acid while levofloxacin and metronidazole were considered for *gyrA* and *RdxA* genes respectively.

2.9.1. Modelling of protein structures

Application of bioinformatics to analysis of 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 and https://yanglab.nankai.edu.cn/trRosetta/for tertiary structure. 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 version 3.1.4.

3. Results

3.1. Study participants' socio-demographic characteristics and study plan

The total recruited study participants were 169. These were patients scheduled for diagnostic endoscopy because of symptoms of dyspepsia. Details of socio-demographic characteristics of study participants are presented in Table 2. They consisted of 50 males and 119 females with an average age of 41 years. About 22.49% of the participants lacked formal education, whilst 40.46% of those who had received formal education had only completed elementary training. The rest had obtained at least secondary school education. Following each patient's consent to take part in the study, antral gastric biopsies were taken. Out of the 169 gastric biopsies obtained, rapid urease test results showed the presence of *H. pylori* infection in 60.95% (N = 103) of the samples. After culturing, isolation of bacteria was successful in a total of fifteen (15) samples (14.56 %) out of the total 103 *H. pylori* positive samples (Fig. 1). Literature search showed that G242C and T254I mutations in *Pbp1* gene have not been detected in earlier studies elsewhere (Fig. 1).

Table 1
List of primer sets used for genotyping *H. pylori* by PCR and their expected sizes.

Gene	Primer sequence (5′ to 3′)	Annealing temperature (°C)	Size (bp)
Hp23Sr6/r7	F: CACACAGGTAGATGAGATGAGTA R: CACA*CAGA*ACCACCGGATCACTA	60	768
DNA gyrase A (*gyrA*)	F: AGCTTATTCCATGAGCGTGA R: TCAGGCCCTTTGACAAATTC	62	582
Pbp1	F: AGCCATTCTTATCGCTC R: CGACTAGCATGGTGATTT	56	612
RdxA	F: GCAGGAGCATCAGATAGTTC R: GGGTGATTTCTTGGTTGC	57	886

Source: (Matteo et al., 2008; Pourakbari et al., 2018; Suzuki et al., 2013; Zhang et al., 2020)

Table 2
Socio-demographic characteristics of study participants.

Characteristic		Frequency	Percentage (%)
	Males	50	29.59
	Females	119	70.41
Age group (years)	30 and below	27	15.98
	31–50	54	32.54
	51–70	68	40.24
	Above 70	19	11.24
Level of education	No formal education	38	22.49
	Elementary training	53	31.36
	Beyond elementary training	78	46.15

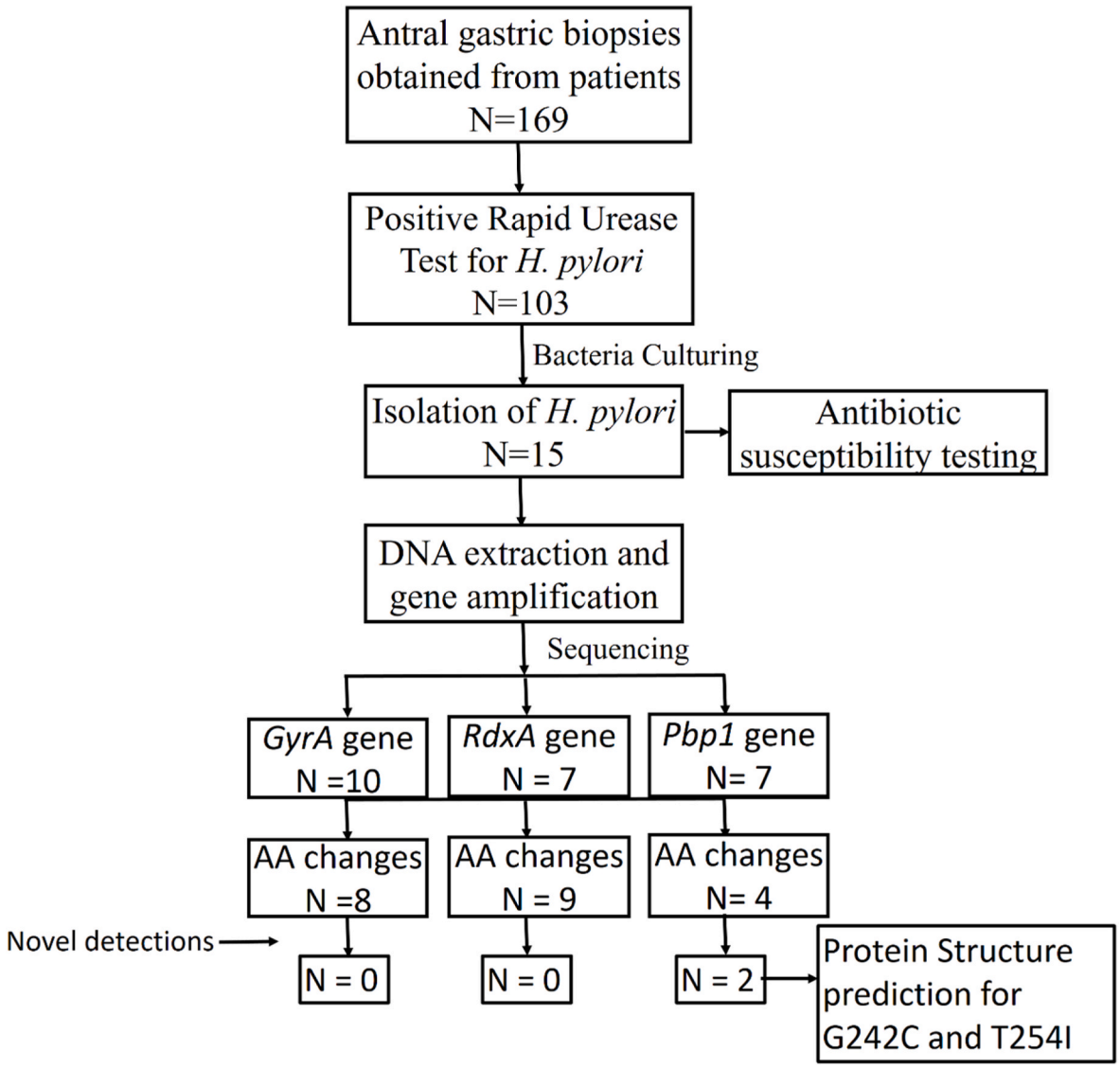

Fig. 1. Study Plan Showing Participants Recruitment and Molecular analysis of *H. pylori* antibiotic resistance in Ghanaian dyspepsia patients. Amino acid (AA) substitutions are observed in GyrA, RdxA and Pbp1 genes at N = 10, 7, and 7 respectively. Non synonymous mutations identified in various genes have been reported in earlier studies elsewhere except G242C and T252I in pbp1 which were not found in our search for existing mutations. Secondary and tertiary structures of these novel genes were modelled for observation of possible structural changes resulting from the changes in amino acids.

3.2. Antibiogram of H. pylori isolates

Metronidazole, amoxicillin, amoxicillin-clavulanic acid and clarithromycin tested against the isolated strains of *H. pylori* showed complete resistance while varied susceptibility was recorded for tetracycline, ciprofloxacin and Levofloxacin (Table 3).

Table 3
Percentage resistant and susceptibility rates obtained for various antibiotics.

Antibiotic tested	Sensitive		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

3.3. Detection of antibiotic resistance genes and H. pylori bacterium by PCR

Electrophoregram of amplified genes for *H. pylori* detection (Fig. 2B), as well as *rdxA*, *pbp1*, and *gyrA* genes are shown in Fig. 2. Amplified PCR products on 2% agarose stained with ethidium bromide were viewed under UV light. Amplicon sizes were determined by comparison with 100bp molecular weight marker (Bioneer cooperation).

In Fig. 2A for presentation of various antibiotic resistance genes, Lanes 1 and 7 represent molecular weight marker used (100bp Bioneer weight marker) while lanes 6 and 12 shows no detection of any of the resistance genes under study. Lanes 2–5 shows the detection of pbp1 gene at 612bp: Lanes 8–11 shows presence of GyrA genes at 582bp and Lanes 13–15 shows RdxA gene detection at 886bp. Quantity of sample used in wells was 5 μL.

Fig. 2B show the bands obtained for the detection of *H. pylori* specific 23sRNA (Hp23sr6/r7) gene. Lanes 3, 4, 9–11, 14, 15 are negative for the presence of *H. pylori* while Lanes 2, 5–8, 12, 13 shows positive results for the detection of *H. pylori*. Lane 1 is molecular weight marker.

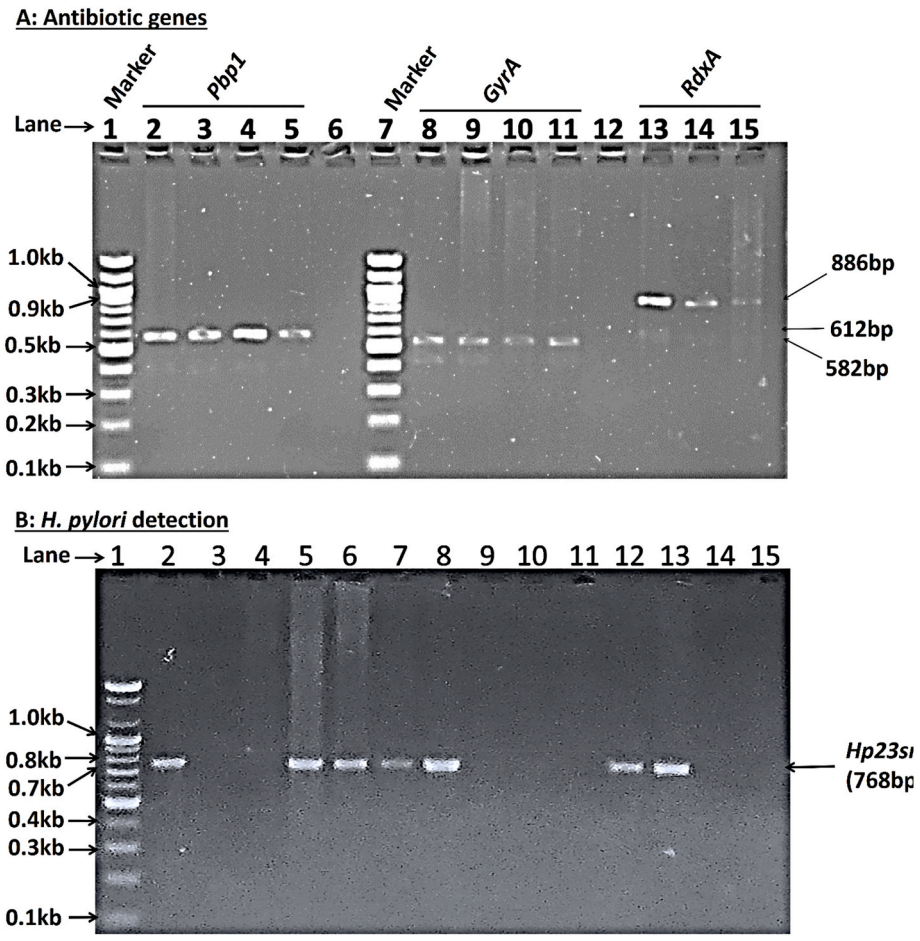

Fig. 2. Electrophoregrams obtained for identification of *H. pylori* and antibiotic resistant genes.

3.4. Phenotypic findings in LEV, MTZ and AMX compared to point mutations

Six (6) of the isolates were resistant to all three antibiotics. Comparable point mutations are observed in Levofloxacin resistant and susceptible isolates with each occurring at not less than four (4) different positions. Amino acid substitutions supporting mutations in the target genes were detected. Some sequences obtained were not appropriate for mutational analysis which brought the sequences to be analysed to ten (10) for *gyrA* and seven (7) for each of *rdxA* and *pbp1*. Base mutations leading to observed point mutations are shown in Table 4. Various non-synonymous mutations were identified in all genes with the highest occurring in *rdxA* (Table 4). Regardless of recording a 100% phenotypic resistance in amoxicillin, point mutations in *pbp1* were observed to be at the lowest (Table 4). All sequenced *rdxA* genes contained Q6H, R90K, G98S, R131K, and A183V point mutations while sequenced *gyrA* gene all contained M191I and G208S. Q50stop was identified for samples Hp 7 and Hp8 for *rdxA* gene. Multiple allelic mutation was identified for all *pbp1* resistance gene sequences at G242 C/A. It is worthy to note that pfam was used to determine the domains of the pbp gene in which the novel mutant was identified (Fig. 7). This gave an idea that the mutations could influence protein folding and eventually affect enzyme function (see Table 5).

3.5. Alignment of sequenced genes for mutation analysis

PCR products that were successfully sequenced were aligned with reference strain using Ugene 45.0 software and base mutations were analysed for possible amino acid substitutions in various genes (Fig. 3). Areas with observed base mutations are shown with black rectangles where base changes are seen when compared to the reference sequence. G1-G10 represents isolates Hp1-Hp10, PB1-PB8 represent isolates Hp1-Hp8, and R1-R12 represent isolates Hp1-Hp12. A number of synonymous mutations were also identified which includes a-d in *gyrA* (Fig. 3A), c-e in *pbp1* (Fig. 3B), and b-d, f, g in *rdxA* (Fig. 3C). In numbering the positions of nucleotides, the nucleotide in the first position is considered to be the first nucleotide of the gene's start codon.

3.5.1. Geographic distribution of detected mutations

Literature search was done to identify earlier studies that have identified mutations found in the current study and presented in Fig. 4. The map shows the geographic distribution of *Helicobacter pylori* mutations identified in the current study, emphasizing the differences in genetic diversity between regions. Mutations or strains found in different places are represented by different colours. Localized adaptations, whether impacted by environmental variables, antibiotic resistance, or human migration patterns, are suggested by clusters of mutations.

3.6. Structure modelling of proteins involving novel mutations identified

In the secondary structures of Figs. 5 and 6, black rectangle in wildtype is compared to black rectangle in mutant structure. Red rectangles in both structures are also compared. In Fig. 6, blue and green rectangles shown in wildtype secondary structure are compared to their respective colours in G2424C mutant secondary structure.

In the tertiary structures of Figs. 5 and 6, cartoon and surface model structures of the wildtype and mutant genes of pbp1 with areas of

Table 4
Point mutations observed in antibiotic resistance genes compared to antibiogram findings.

Isolate	Susceptibility (phenotypic)			Point mutation		
	LEV	MTZ	AMX	*GyrA*	*RdxA*	Pbp1
Hp1	**S**	**R**	**R**	N87I, A97V, M191I, V199A, H200Y, G208E	Q6H, R90K, G98S, R131K, A183V	G242C, G242A, T254I, S417T
Hp2	**R**	**R**	R	N87T, M191I, V199A, G208E	**ND**	G242C, G242A, S417T
Hp3	**R**	**R**	R	N87I, M191I, V199A, G208E	**ND**	G242C, G242A
Hp4	**R**	**R**	**R**	N87I, V199A M191I, G208E	Q6H, R90K, G98S, R131K, A183V	**ND**
Hp5	**R**	**R**	**R**	N87I, M191I, V199 M, G208E	K2N, Q6H, R90K, G98S, R131K, A183V	G242C, G242A
Hp6	**S**	**R**	**R**	N87I, M191I, V199A, G208E	K2N, Q6H, R90K, G98S, E75K, R131K, A183V	G242C, G242A
Hp7	**R**	**R**	**R**	N87I, V199A, M191I, G208E	Q6H, Q50X, R90K, G98S, R131K, A183V	G242C, G242A
Hp8	**S**	**R**	**R**	N87T M191I, V199A, G208E	Q6H, Q50X, R90K, G98S, R131K, A183V	G242C, G242A
Hp9	**S**	**R**	**R**	N87I, M191I, V199A, G208E	**ND**	**ND**
Hp10	**R**	**R**	**R**	N87I, M191I, V199A, G208E	**ND**	**ND**
Hp11	**S**	**R**	**R**	**ND**	**ND**	**ND**
Hp12	**S**	**R**	**R**	**ND**	K2N, Q6H, R90K, G98S, E75K, R131K, A183V	**ND**

ND, not determined (the obtained sequence was not appropriate for mutational analysis); R = resistant and S = sensitive. LEV, MTZ and AMX represent levofloxacin, metronidazole and amoxicillin respectively.

Table 5
Base mutations and their resultant point mutations for *gyrA*, *pbp1*, and *rdxA* genes.

Gene	Base mutation	Point mutation
GyrA	AAT → ATT	N87I
	AAT → ACT	N87T
	GCG → GTG	A97V
	ATG → ATT	M191I
	GTG → ATG	V199 M
	GTG → GCG	V199A
	CAT → TAT	H200Y
	GGA → GAA	G208E
Pbp1A	GGC → TGC	G242C
	GGC → GCC	G242A
	ACC → ATC	T254I
	TCT → ACT	S417T
RdxA	AAA → AAT	K2N
	CAG → CAC	Q6H
	CAG → TAG	Q50X
	GAA → AAA	E75K
	AGA → AAA	R90K
	GGC → AGC	G98S
	CAT → CCT	H99P
	AGA → AAA	R131K
	GCA → GTA	A183V

structural modifications due to the mutation are highlighted with squares. Here, Black Square in wildtype of cartoon structure (A) is compared to black square in mutant (B) and merged cartoon structures (C) (Figs. 5 and 6). Again, green and yellow squares in wildtype of cartoon structure is compared to their respective colours in mutant and merged cartoon structures (Fig. 5). Similarly, black square in surface structure for wildtype (A) is compared to black squares in mutant (B) and merged surface structures (C) (Fig. 5).

From the secondary structure, a helix (shown with black rectangle) in the wildtype is observed to have shortened in the T254I structure (shown in black rectangle). At positions 543 and 544 (shown with red rectangle), the amino acids S and L which make up a helix in the wildtype is observed as coils in the T254I mutant secondary structure.

Tertiary structures also show a shortened helix in T243I mutant (shown with yellow square) in the cartoon structure and non-aligning helix shown with black and green squares. The non-alignment of helix is observed in surface structures (shown with black squares)

Secondary structures show an additional amino acid (D at position 40) adding up to a helix that originally comprised of amino acids from positions 35–39 (shown with black rectangles). A helix at 43–44 (shown with green rectangle) in the wildtype is also observed as a coil in the mutant. Also, a coil in the wildtype (shown with blue rectangle) is observed as a strand in the mutant.

Tertiary structures show non-alignments in cartoon and surface structures. A helix in the wildtype cartoon structure (A) is observed to be shortened in the mutant (B) (shown with red squares) as seen in the merged (C)

4. Discussion

Treatment for *H. pylori* infection has continuously proven difficult, but combining antibiotics into one regimen has demonstrated to be an effective alternative approach (Pohl et al., 2019). A combination of amoxicillin, clarithromycin, and metronidazole along with a proton pump inhibitor is the recommended treatment for *H. pylori* infection in Ghana, as per the Standard Treatment Guidelines (2017). However, this regimen is waning in efficacy, likely resulting in the unresolved or recurrent symptoms among many suffering from this infection (Jaka et al., 2018). A successful course of treatment minimizes the risk of gastric malignancies, and reduces the economic burden of equally severe *H. pylori*-related diseases (Suzuki and Mori, 2018). These factors notwithstanding, 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 suggests that studies conducted elsewhere may have served as the foundation for the current treatment plan, which may not be very successful against the strains found in Ghana. Again, 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). Variations exist in strains and their characteristics across different locations which also affects the pathologies that may be presented (Thorell et al., 2023). Antimicrobial resistance, mainly caused by mutations in target genes, is recognized as a major 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. The core of the molecular mechanisms involved in this infection have not been fully understood and researchers continue to fill in the voids. Therefore, there is the need to understand the mechanism of bacterial resistance to the current antibiotics as an important step to informing future treatment guidelines.

Mutations mainly result in modifications of antibiotic target sites and/or reduced membrane penetrability, affecting the activity of the

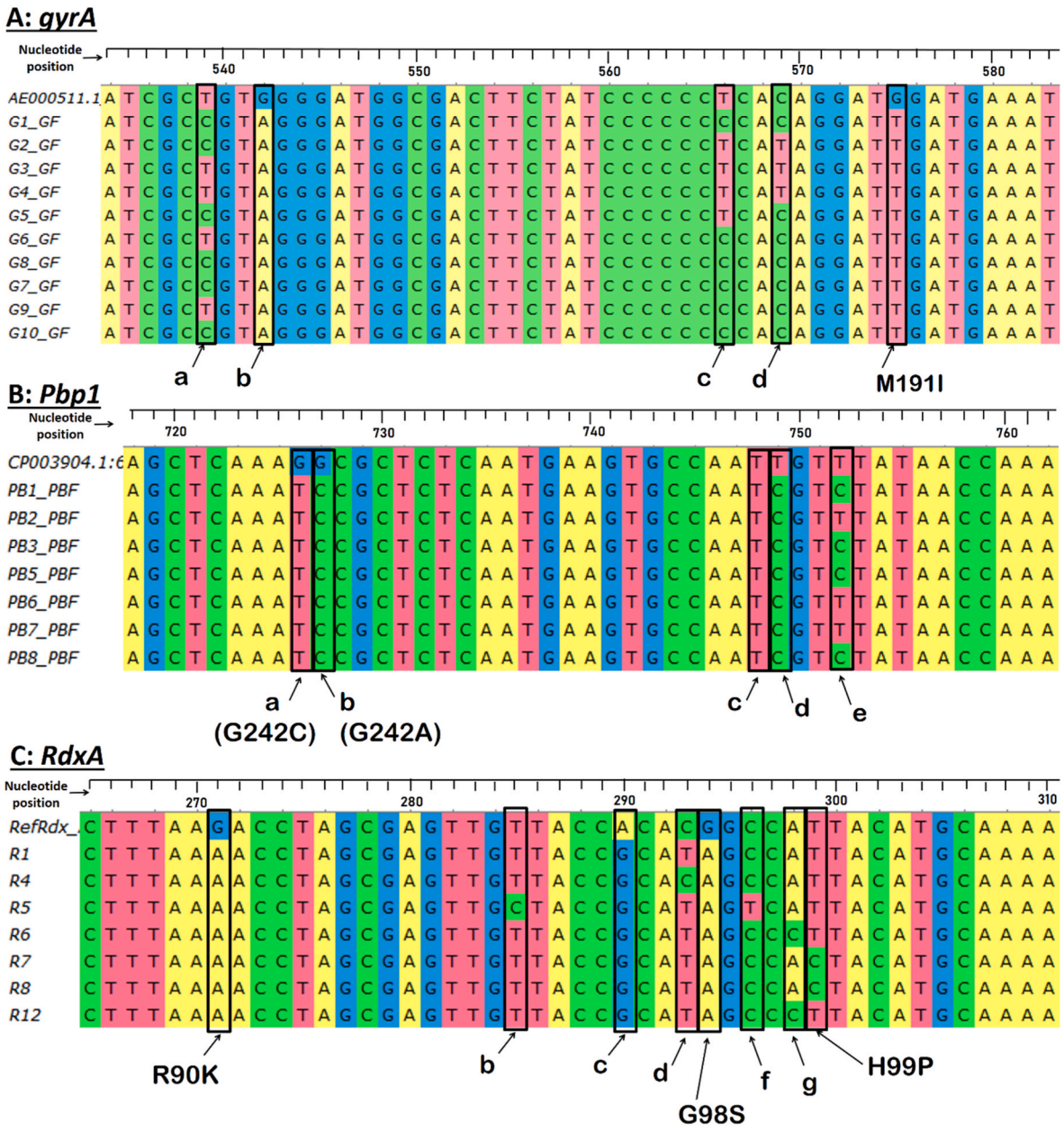

Fig. 3. Alignment of antibiotic resistance genes with reference. *Nucleotide substitution of G for T resulting in point mutation M191I in gyrA gene is shown in A; B shows alignment of pbp1 gene in base changes at position 242 (with codon GGC) results in point mutations G242C and G242A observed for all sequences analysed. Point mutations R90K and G98S were recorded for all samples whereas isolates Hp7 and Hp8 recorded H99P when rdxA genes were aligned (shown in C). All other base mutations resulted in synonymous mutations.*

drug (King, 2021). Therapeutic failures in fluoroquinolone drugs (eg. levofloxacin) are principally by the inhibition of DNA gyrase (topoisomerase II) enzyme encoded in distinct regions like the *gyrA* gene. Point mutations in sections encoding amino acids at positions 86–88, 91, 97, or 130 have been widely associated with levofloxacin resistance with 87, 88 and 91 regarded as hot spot positions (Bilgilier et al., 2021; Bogaerts et al., 2006; Fernández-Caso et al., 2023; Wu et al., 2012). Point mutations observed in *gyrA* gene for the current study included N87T, N87I, A97V, M191I, V199 M, V199A, H200Y, and G208E. Each isolate, including sensitive strains recorded at least four (4) different point mutations with M191I and G208E occurring in all. Sensitive strains showed no striking difference in terms of point mutations when compared to resistant strains. A study by Farzi et al. (2019)(Farzi et al., 2019) found M191I, V199A, G208E and A97V to be present in both resistant and susceptible strains of Iranian *H. pylori* isolates. 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 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 as well as A199V/I, R103H, H57Y, S63P, A88 N/P/V, V65I, V77A, S83A, D86 N, R130K, N87 A/K/I/Y/T, D91 G/N/A/H/Y, D161 N, A92T, D99V, A129T, D155 N, V172I, P188S, D192 N) (Tshibangu-Kabamba and Yamaoka, 2021).

Metronidazole is a pro-drug that requires enzyme activation to perform its function. This activation occurs in *H. pylori* cells when ferredoxin or ferrodoxin oxidizes metronidazole by the donation of electrons from pyruvate oxidoreductase complexes (Arslan et al., 2017; Garrido-Treviño et al., 2022). As a result, nitro groups are reduced into

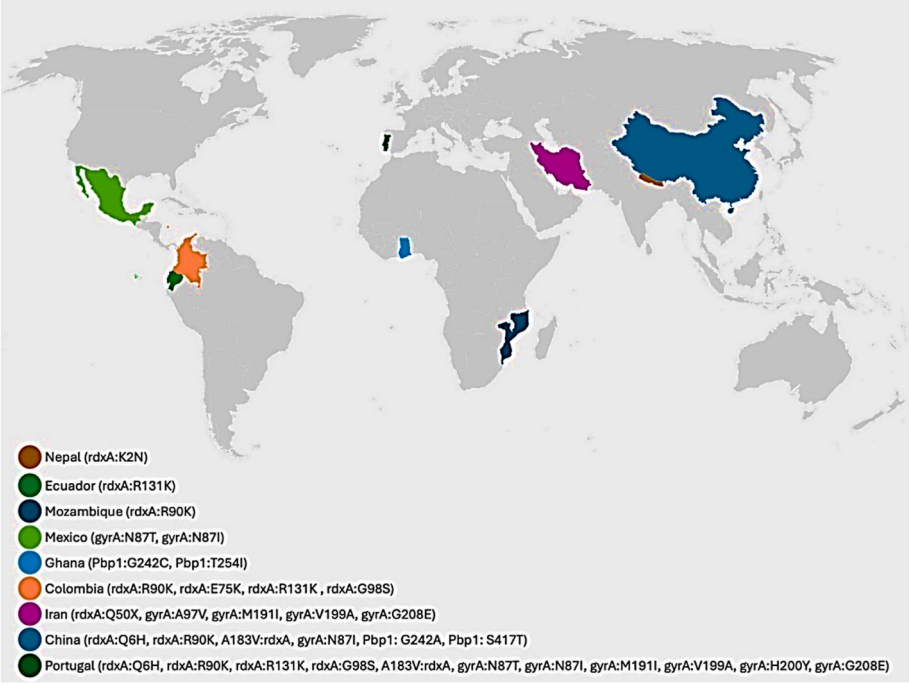

Fig. 4. Geographic distribution of mutations identified in the current study.

forms that can prevent the synthesis of nucleic acid. A decline in activity of nitroreductase enzymes is caused by mutations in oxidoreductases, such in the *rdxA* gene (oxygen-insensitive NADPH nitroreductase). This impact slows down the rate at which metronidazole activates inside the bacterium, which leads to antibiotic resistance (Garrido-Treviño et al., 2022; Megraud, 1997). Earlier studies have demonstrated association of metronidazole resistance to mutations in *RdxA* gene at positions R16 C/H, Y103H, and S121D (King, 2021; Li et al., 2022). In the current study, all isolates proved phenotypically resistant to metronidazole with at least six (6) different point mutations. Analysis of *RdxA* gene sequences showed a number of amino acid substitutions including K2N, Q6H, E75K, R90K, G98S, H99P, R131K, and A183V. Our study also found point mutations introducing a stop codon at position Q50Stop for two (2) isolates. This represents an early termination of protein synthesis that can result in *rdxA* protein non-functionality or redundancy. It is to be noted however that mutations in only *rdxA* genes for metronidazoles resistance are unable to explain MTZ resistance in *H. pylori* isolates making it important to investigate additional mechanisms that may be contributing to this challenge according to a study by Marques et al. (2019), Ferredoxin-like protein (*fdxB*) and NADPH flavin oxidoreductase (*frxA*) are other nitroreductases that require more research since they may contribute to low rate metronidazole resistance in *H. pylori* (King, 2021; Tanih et al., 2010). *RdxA* gene could impact activities of the gene including de novo inactivation which can lead to a damage to the helical structure of DNA by reducing hydroxylamine (Goodwin et al., 1998). Once these genes are deactivated, they are unable to encode the enzymes required to reduce the pro-drug metronidazole nitro groups into their bactericidal agent as a result of a change in activity (King, 2021).

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 (Garrido-Treviño et al., 2022; Saracino et al., 2021). The most common mutations identified in amoxicillin resistant strains are substitutions at positions N562Y, T556S, and S414R(Rimbara et al., 2008). This study recorded isolated *H. pylori* strains each possessing multiple allelic variants of G242C and G242A. All isolates were phenotypically resistant to amoxicillin. Point mutation at T254I was observed for Hp1 isolate while S417T was found in both Hp1 and Hp2 isolates. 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; D535 N, F366L, V45I, S338R, V374L, S402G, N404S, S405 N, S414R, L423F, S455 N, 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 and Yamaoka, 2021). In our literature search, it has been realized that point mutations at T254I and G242C have not been detected elsewhere and are therefore novel. Structural modelling of these two variants shows their ability to cause modification of the PBP proteins which could affect the binding action of the drug to the organism thereby inhibiting the treatment potential of amoxicillin. Two key enzymatic activities involving transglycosylase and transpeptidase play a crucial role in bacterial cell wall synthesis of *H. pylori*. These enzymes make up the domains of the gene with their start to end amino acid positions being 20–197 for transglycosylase and 298–550 for transpeptidase. Mutations relating to G242C and T254I which are found outside the domains are expected to cause an effect in the overall protein folding and eventually a possible effect on the activity of the enzymes.

Although amoxicillin-clavulanic acid combination is reported to produce higher inhibitory activity against *H. pylori* infection (Alrabadi et al., 2021; Dehghani et al., 2009), the present study indicates a 100 % phenotypic resistance. This study may therefore serve as grounds for further scrutiny and a possible second look at the antibiotics of choice against the infection in Ghana. The findings indicate that standard triple therapy comprising of MTZ with AMX or CLR may not be ideal for consideration as first-line treatment. Findings in the phenotypic resistance of our study is comparable to those of Kouitcheu et al. (2019), and Chukwudike et al. (2021). Phenotypically, average resistance rate detected elsewhere for 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: 74.1-0.77.4) respectively(Jaka et al., 2018). Resistance to amoxicillin is not as widespread as that of metronidazole and a number of studies have reported low resistance rates to amoxicillin and tetracycline (Diab et al.,

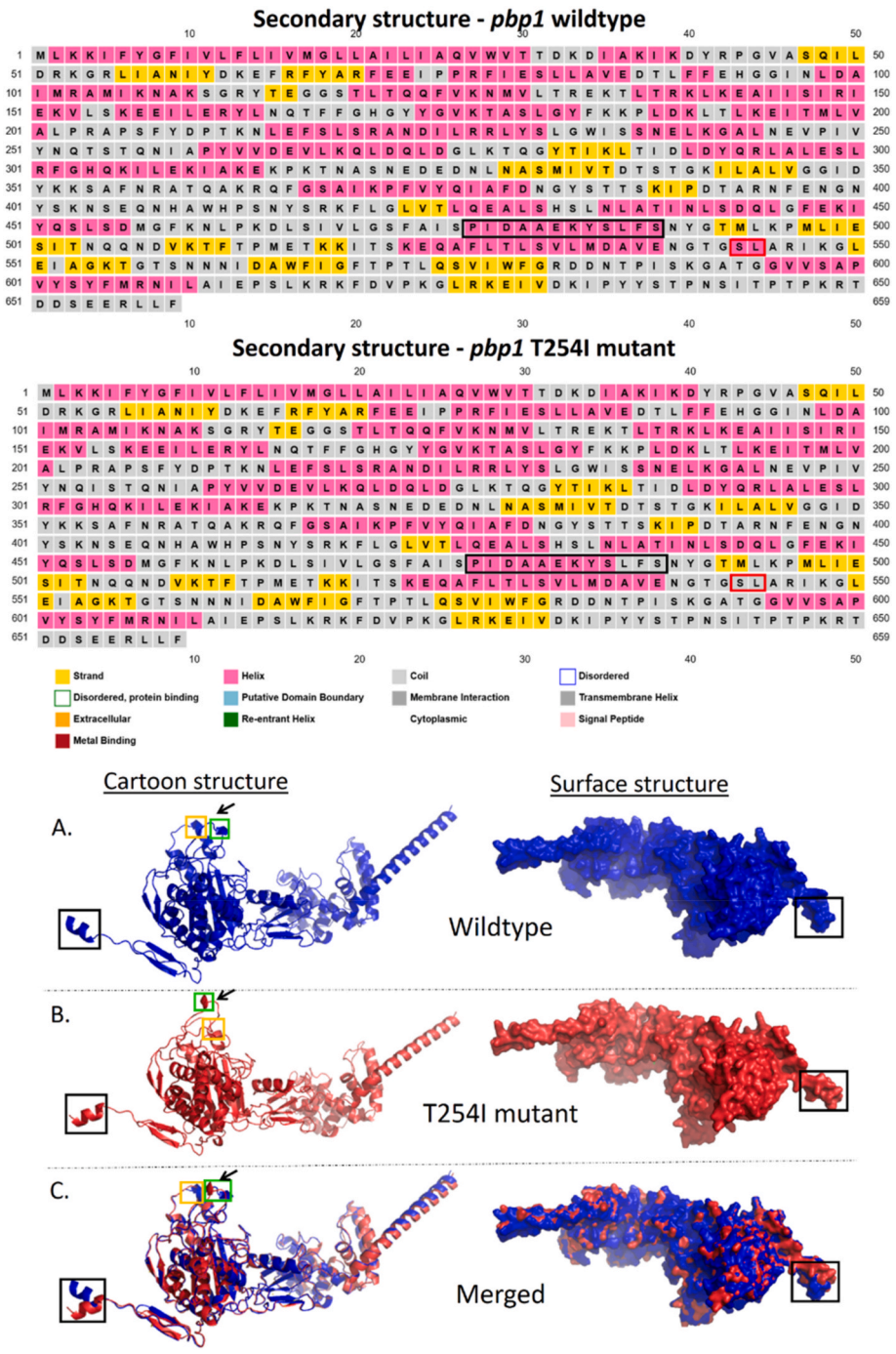

Fig. 5. Secondary and tertiary structures of wildtype and mutant T254I *pbp1* mutant genes.

2018). However, in Nigeria, a rate as high as 100 % has been observed (Aboderin et al., 2007) 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 the treatment of a variety of infections (D'Arcy et al., 2021). The frequent prescription of these drugs as common anti-microbial agents could contribute to the high level of resistance in *H. pylori*. Phenotypic observations indicate 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. Our research shows that traditional first-line antibiotics are often ineffective against several clinical isolates of *H. pylori* due to resistance. Identifying antibiotics that still work, like tetracycline and ciprofloxacin, allows healthcare providers to make more informed choices regarding treatment plans. Since *H. pylori* infections are a significant global health issue associated with gastric disorders, recognizing local resistance patterns can guide public health initiatives and antibiotic usage strategies.

5. Conclusion

Our findings reveal that *H. pylori* in Ghana exhibits significant resistance to metronidazole, amoxicillin, and fluoroquinolones, highlighting the need for alternative treatment strategies for eradication therapy. The resistance rates to metronidazole, amoxicillin, amoxicillin-clavulanic acid, and clarithromycin are notably high in the study area. In

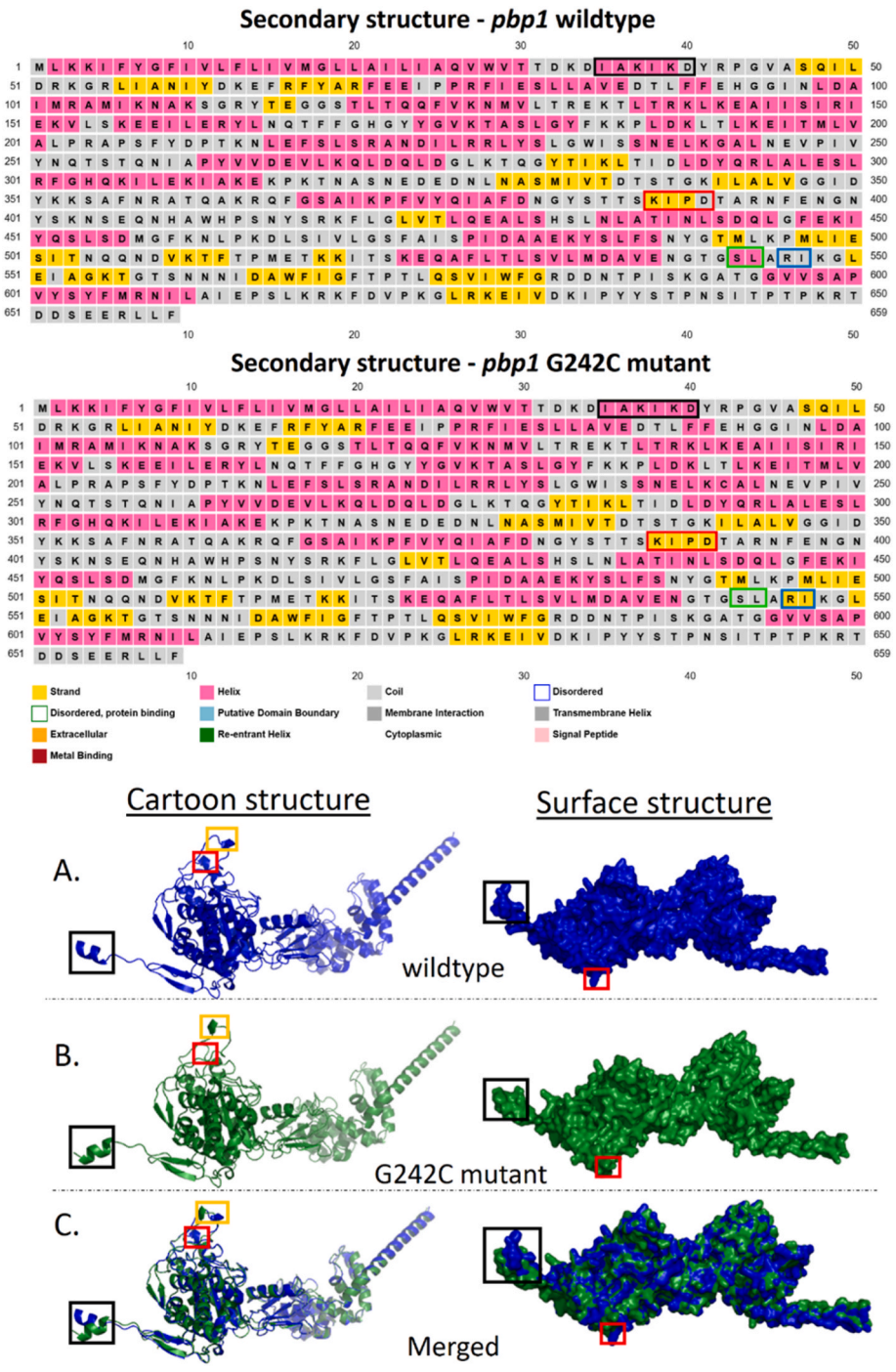

Fig. 6. Secondary and tertiary structures of wildtype and mutant G242C *pbp1* mutant genes.

contrast, ciprofloxacin, levofloxacin, and tetracycline show relatively better susceptibility, making them more viable treatment options. Additionally, mutations in the target genes for metronidazole, levofloxacin, and amoxicillin may contribute to drug resistance in *H. pylori* in Ghana. Importantly, testing for *H. pylori* antibiotic resistance before initiating treatment is essential. The most effective approach to reducing resistance rates may involve streamlining eradication programs and minimizing the overall use of antibiotics in the population.

CRediT authorship contribution statement

Eric Gyamerah Ofori: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Foster Kyei:** Writing – review & editing, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis. **Emmanuel Ayitey Tagoe:** Writing – review & editing, Supervision, Methodology, Investigation, Formal analysis, Data curation. **Ansumana Sandy Bockarie:** Writing – review & editing, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Samuel Mawuli Adadey:** Writing – review & editing, Visualization, Software, Investigation, Formal analysis, Data curation. **Osbourne Quaye:** Writing – review & editing, Validation, Supervision, Methodology, Data curation. **Michael Buenor Adinortey:** Writing – review & editing, Visualization, Supervision, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Gordon Akanzuwine Awandare:**

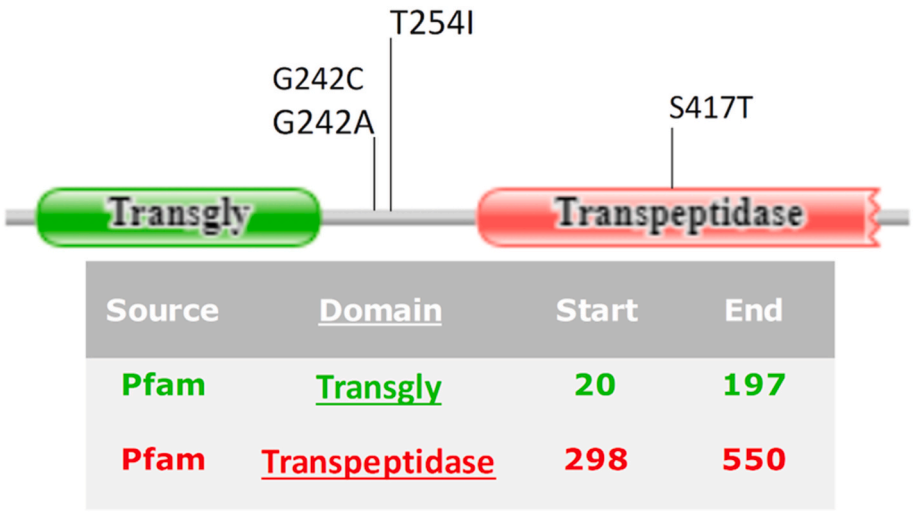

Fig. 7. Domains of the pbp gene identified using pfam.

Writing – review & editing, Software, Resources, Project administration, Methodology, Funding acquisition. **Cynthia Ayefoumi Adinortey:** Writing – review & editing, Visualization, Supervision, Methodology, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that there is no conflict of interest or personal relationships that might influence the work reported in this paper.

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