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FREQUENCY OF ANTIBIOTIC RESISTANCE MUTATIONS IN GYRA, GYRB, AND FRXA OF HELICOBACTER PYLORI

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ABSTRACT
Helicobacter pylori infect about 50% of the world's population and it's considered as the first etiological agent of severe gastric diseases, such as peptic ulcers and gastric carcinoma. Increasing resistance to standard antibiotics led to decreasing efficacy of bacterial eradication. Levofloxacin and metronidazole are considered as a part of the eradication regimen of H. pylori and resistance to these drugs is in increase. This is a prospective cross-section study included a total of 100 specimens of gastric tissue biopsies(GTBs) from patients diagnosed with different gastro-duodenal diseases. Molecular identification of H. pylori, molecular detection of cagA and amplification of specific fragments in gyrA ,gyrB , frxA in directly in extracted DNA from GTBs were performed via conventional PCR. Partial sequencing of gyrA ,gyrB and, frxA amplicons of 9/100 specimens was performed via Sanger sequencing. The results showed that 82/100 (82%) specimens were positive for H. pylori and 20/82(24.39%) of specimens were positive for cagA. Analysis of partial sequencing of gyrA and gyrB amplicons showed a high frequency of synonymous nucleotide changes and two strains were carried (C/T at position 130) in gyrA which confirmed as resistance mutation. A high frequency of missense mutations and frame-shift mutations was detected in frxA. In conclusion, the effect of these nucleotide changes is not conformed because of some limitation in the study but it give indication that these changes may have an effect in the affinity and the effectiveness of antibiotic to eradicate the bacteria.

Index Terms – frxA, gyrA-gyrB, Helicobacter pylori, Partial sequencing, Resistance mutations.

INTRODUCTION
Helicobacter pylori infection is one of the most prevalent "infectious diseases" in the world. Approximately, 50% or more of the world's population having *H. pylori* in their stomachs [1],[2]. Drug resistance is recognized as one of the greatest public health threats, leading to 700,000 deaths per year worldwide. *Helicobacter pylori* has emerged as an alarming bacterium due to its resistance rate, and it has been noted as one of 16 antibiotic-resistant pathogens that pose the most serious threat to human health, according to World Health Organization (WHO), since 2017 [3]. Due to widespread antimicrobial resistance, the first-line treatment for *H. pylori* infections has a decreasing cure rate. In contrast, a chronic infection can lead to the development of gastro-duodenal diseases such as peptic ulcer and stomach cancer. A recent agreement on *H. pylori* treatment strongly advocated modifying the treatment based on the findings of antimicrobial susceptibility testing to address the issue[4]. Eradication of *H. pylori* is not an easy task. Historically, a single drug is not effective in eradicating *H. pylori.* Only a few antibiotics, such as Levofloxacin, metronidazole, amoxicillin, and clarithromycin, are effective in eradicating *H. pylori*. A typical treatment regimen usually consists of two or three of these antibiotics, in combination with anti-acids and/or bismuth. The availability of limited effective treatment options and widespread use of some antibiotics in the population (such as quinolones for infectious diarrhea), coupled with the adaptability of the species, were causing an increase in *H. pylori* resistance [5].

Levofloxacin, a fluoroquinolone, target bacterial chromosome replication, in particular, DNA gyrase, which allows DNA unraveling before replication. Resistance to Levofloxacin has been mainly associated with point mutations occurring at positions Asn87 and Asp91 of the quinolone resistance determining region (QRDR) within *gyrA*. About 14 Amino acid substitutions at positions 91 (D91G, N, A, Y or H) and 87 (N87L, I, A or K) of *gyrA* were most frequently associated with levofloxacin resistance. The changes in the *gyr*B, mainly at position438, 463, 484 also should be taken into consideration [6].

Metronidazole, a nitro-imidazole, acts as a biocidel agent by its interaction with a nitro-reductase homolog. Mutations in *rdxA* were shown to be the causes of *H. pylori* resistance to metronidazole. Also, mutations in *frxA*, which encoding to NADH flavin oxido-reductase, was implicated in *H. pylori* metronidazole resistance[7]. Most metronidazole-resistant *H. pylori* strains carry multiple mutations in r*dxA* and *frxA*. In particular, frame-shift mutations (i.e., at codon positions 105, 149 or 192 in *frxA* and 18, 38 and 112 in *rdxA*) and point mutations resulting in amino acid exchanges (i.e., A67V, A68E, K64N, P106S, R90S and R16C/H in *rdxA*) were observed only in metronidazole resistant strains. Other mutations (i.e., at codon positions 18 in *frxA* and 62, 96 and 162 in *rdxA*) were distributed between resistant and susceptible strains[8].

The current study was aimed to detect the presence of suspected resistant mutations to levofloxacin and metronidazole in *gyrA,gyrB* and *frxA*, respectively, directly in extracted DNA from GTBs of patients with *H. pylori* infections and to determine the frequency of antibiotic resistance mutations in these genes in association with clinical presentation of patients, in order to applied direct molecular identification of bacteria and susceptibility to the most important antibiotics used in treatment of *H. pylori* as routine test in bacteriology labs.

METHODOLOGY

A. Sampling:

The current cross-sectional study was involved 100 patients suffering from gastro-duodenal manifestations (dyspeptic symptoms, abdominal pain, nausea, and vomiting) recruited to Gastro-Endoscopy Department at Gastro-enterology and Hepatology Teaching Hospital, Baghdad, Iraq. According to consultant physician instructions, those patients were submitted to clinical examination and endoscopy. The range of patient's age was 18 years-65 years. Male to female ratio was 1:1. Two gastric tissue biopsies (GTBs) were collected from each patient undergoes to endoscopy, one was placed in 10% formalin and sent to histo-pathological laboratory and the second one was placed in 1ml of normal saline and preserved at -20°C for molecular analysis. Histo-pathology findings were collected from patient's laboratory reports. DNA extracted from GTB samples obtained from patient with normal mucosa was used as negative control for *H. pylori* infection in molecular studies.

B. Inclusion and exclusion criteria

Patients with different gastro-duodenal manifestations were included in the current study. Patients under 18 years old , patients with a history of gastric surgery or active gastrointestinal bleeding and pregnant female patients were excluded from study.

C. Ethical considerations

This study was approved by the Institutional Review Board of Al-Nahrain University-College of Medicine (IRB/2867/3/2)**.**

D. Molecular identifications of Helicobacter pylori and cagA

DNA was extracted from fresh GTBs using Wizard® Genomic DNA Purification Kit(Cat. No A1120,USA) according to manufacturer instructions. Direct molecular identification of *Helicobacter pylori* in extracted DNA from GTBs using primer set for identification of species-specific *ureA* in *H. pylori* [9]. Direct molecular identification of Cytotoxin-associated gene A in extracted DNA from GTBs was performed using conventional PCR[10]. Amplification of target fragment in *gyrA , gyrB* and *frxA* in extracted DNA was performed[7,11].

E. Partial sequencing of amplified target sequences in gyrA , gyrB and frxA

Farther study of hot spot resistance mutations to Levofloxacin and Metronidazole were performed via sending amplicons of target sequences of *gyrA ,gyrB* and *frxA* from 9 selected *H. pylori*+ve DNA samples for Sanger sequencing using automated DNA sequencer ABI 3730XL (Macrogen Corporation, Korea). The results were analyzed using Genious software. The sequences of each fragment were trimmed to a uniform length that corresponded with the region used to identify the target gene. Sequences were compared with standard strains using online BLAST software (http: //www.ncbi.nlm.nih.gov/BLAST/).

Statistical analysis

Data were collected, summarized, analyzed and presented using statistical package for social sciences (SPSS) version 23 and Microsoft Office Excel 2010. Qualitative (categorical) variables were expressed as number and percentage. Fischer exact test was use to evaluate association between any two categorical variables. The level of significance was considered at P-value of equal or less than 0.05.

RESULTS

A. Direct molecular identifications of Helicobacter pylori and cagA

Results of direct molecular identification of *H. pylori* in 100 extracted DNA from GTBs showed that 82/100(82%) of samples were positive. Results of direct molecular detection of *cag* A in 82 extracted DNA samples from GTBs showed that 20/82(24.39%) of extracted DNA samples were positive.

B. Partial sequencing of amplified target sequences in gyrA,gyrB and frxA to detect resistance mutations

Several of synonymous and non- synonymous nucleotide changes were detected in *gyrA*, *gyrB* and *frxA* in comparison with with different stander strains for each sample. Some nucleotide changes in *gyr*A, *gyr*B and *frxA* of *H. pylori* strains were detected in multiple samples from different patients even though each one of the selected samples was blasted with different *H. pylori* stander strain(s), Tables I,II and III, respectively.

Table I. Frequency of Mutations Among Strains Sharing the Same Mutations in gyrA.

***gyr*A**				
Nucleotide change - position	**Frequency of Nucleotide change In studies strains**		**Strain ID**	**Histo-pathological and clinical presentation of patients from who GTB samples were obtained**
	Synonymous	**Non-Synonymous**		
C/T -58	4	NA	HP-11	*H. pylori* gastritis .
			HP-31	*H. pylori* gastritis/abdumanal mass.
			HP-83	*H. pylori*/Gastric polyp
			HP-112	*H. pylori* /Normal mucosa.
T/C-392	3	NA	HP-11	*H. pylori* gastritis.
			HP-36	*H. pylori* gastritis/gastric polyp/ulceration.
			HP-53	*H. pylori* /normal mucosa.
G/A-337	3	NA	HP-11	*H. pylori* gastritis.
			HP-80	*H. pylori* /active chronic gastritis/ intestinal metaplasia/no malignancy.
			HP-112	*H. pylori* /normal mucosa.
T/C-501	NA	3	HP-33	*H. pylori* /normal mucosa.
C/T-501	NA	1	HP-31	*H. pylori* gastritis /abdominal mass.
			HP-36	*H. pylori* gastritis/gastric polyp/ulceration.
			HP-53	*H. pylori* /normal mucosa
G/A-55	3	NA	HP-11	*H. pylori* gastritis
			HP-80	*H. pylori* /active chronic gastritis/ intestinal metaplasia/no malignancy.
C/T-25	2	NA	HP-31	*H. pylori* gastritis /abdominal mass.
			HP-80	*H. pylori* /active chronic gastritis/ intestinal metaplasia/no malignancy.
			HP-112	*H. pylori* /normal mucosa.
A/G-181	3	NA	HP-33	*H. pylori* /normal mucosa.
			HP-69	*H. pylori*/gastric adenocarcinoma cancer .

A/C-34		2	HP-33	*H. pylori* /normal mucosa.
			HP-112	*H. pylori* /normal mucosa .
T/C-364	2	NA	HP-36	*H. pylori* gastritis /gastric polyp/ulceration.
			HP-53	*H. pylori* /normal mucosa
C/T-409	2	NA	HP-36	*H. pylori* gastritis /gastric polyp/ulceration.
			HP-53	*H. pylori* /normal mucosa.
T/C-439	2	NA	HP-36	*H. pylor*i gastritis /gastric polyp/ulceration.
			HP-53	*H. pylori* /normal mucosa.
C/T-46	3	NA	HP-36	*H. pylori* gastritis /gastric polyp/ulceration.
			HP-53	*H. pylori* /normal mucosa.
			HP-69	*H. pylori*/ gastric adenocarcinoma cancer.
C/T-130	2	NA	HP-36	*H. pylori* gastritis /gastric polyp/ulceration.
			HP-53	*H. pylori* /normal mucosa.
C/T-247	2	NA	HP-31	*H. pylori* gastritis/abdominal mass.
			HP-33	*H. pylori* /normal mucosa.
T/C-106	2	NA	HP-33	*H. pylori* /normal mucosa.
			HP-69	*H. pylori* /gastric adenocarcinoma cancer.

-NA: not identified.

Table II. Frequency of Mutation Between Strains Sharing the Same Mutation of gyrB.

gyrB				
Nucleotide change-position	**Frequency of Nucleotide changeIn studies strains**		**Histo-pathological and clinical presentation of patients from who GTB samples were obtained**	
	Synonymous	**Non-Synonymous**	**Strain ID**	**Clinical data**
T/C-7	2	NA	HP-11	*H. pylori* gastritis.
			HP-80	*H. pylori* /active chronic gastritis/ intestinal metaplasia/no malignancy.
C/T-7	3	NA	HP-33	*H. pylori* /normal mucosa.
			HP-83	*H. pylori*/gastric polyp
			HP-112	*H. pylori* /normal mucosa.
C/T-49	2	NA	HP-11	*H. pylori* gastritis.
			HP-112	*H. pylori* /normal mucosa.
A/G-55	2	NA	HP-11	*H. pylori* gastritis.
			HP-33	*H. pylori* /normal mucosa.
T/C- 100	3	NA	PH-11	*H. pylori* gastritis.
			HP-69	*H. pylori*/ Gastric adenocarcinoma cancer.
			HP-80	*H. pylori* /active chronic gastritis/

				intestinal metaplasia/no malignancy.
A/G-445	7	NA	HP-11	*H. pylori* gastritis.
			HP-31	*H. pylori* gastritis/ abdominal mass
			HP-33	*H. pylori* /normal mucosa.
			HP-69	*H. pylori*/ Gastric adenocarcinoma cancer.
			HP-80	*H. pylori* /active chronic gastritis/ intestinal metaplasia/no malignancy.
			HP-83	*H. pylori*/gastric polyp
			HP-112	*H. pylori* /normal mucosa.
C/T-10	1	NA	HP-31	*H. pylori* gastritis/ abdominal mass
T/C-10	3	NA	HP-69	*H. pylori*/ Gastric adenocarcinoma cancer.
			HP-83	*H. pylori*/gastric polyp
			HP-112	*H. pylori* /normal mucosa.
T/C-214	1	NA	HP-33	*H. pylori* /normal mucosa.
C/T-214	2	NA	HP-53	*H. pylori* /normal mucosa.
			HP-83	*H pylori* /gastric polyp.

-NA: not identified.

Table III. Frequency of Mutation between Strains Sharing the Same Mutation of frxA.

frxA				
Nucleotide change-position	**Frequency of Nucleotide change In studies strains**		**Histo-pathological and clinical presentation of patients from who GTB samples were obtained**	
	Synonymous	**Non-Synonymous**	**Strain ID**	**Clinical data**
T/A-22	NA	2	HP-11	*H. pylori gastritis*
			HP-112	*H. pylori /normal mucosa*
A/T-22	NA	1	HP-33	*H. pylori /normal mucosa.*
A/G-190	NA	1	HP-69	*H. pylori/gastric adenocarcinoma cancer.*
G/A-190	1	NA	HP-112	*H. pylori /normal mucosa*
G/A-193	NA	1	HP-69	*H. pylori/gastric adenocarcinoma cancer*
A/G-193	1	NA	HP-112	*H. pylori /normal mucosa*
C/T-313	2	NA	HP-11	*H. pylori gastritis.*
			HP-83	*H. pylori /gastric polyp.*
T/C-313	NA	1	HP-69	*H. pylori/gastric adenocarcinoma cancer*
A/G-319	NA	1	HP-69	*H. pylori/gastric adenocarcinoma cancer*
G/A-319	1	NA	HP-83	*H. pylori /gastric polyp.*
T/C-427	2	NA	HP-11	*H. pylori gastritis*
			HP-	*H. pylori /normal mucosa*

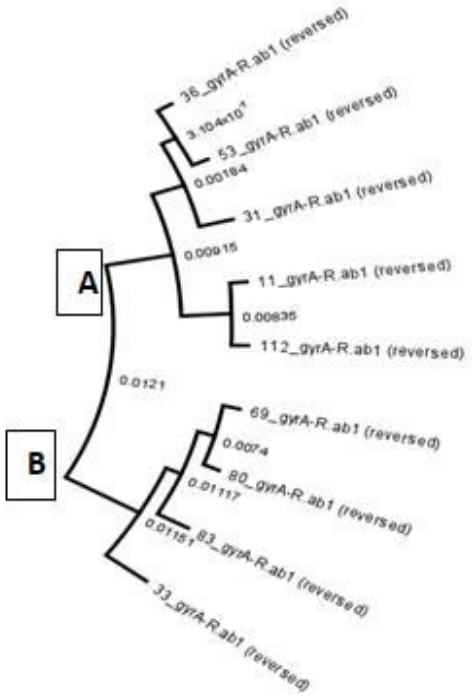
			112	
C/G-589	NA	1	HP-69	*H. pylori/gastric adenocarcinoma cancer*
C/G- 589	1	NA	HP-83	*H. pylori /gastric polyp .*
G/A-592	NA	1	HP-69	*H. pylori/gastric adenocarcinoma cancer*
G/A-592	1	NA	HP-83	*H. pylori /gastric polyp.*
26-28	NA	2	HP-11	*H. pylori gastritis*
			HP-69	*H. pylori/gastric adenocarcinoma cancer*
145	NA	3	HP-11	*H. pylori gastritis.*
			HP-83	*H. pylori/gastric polyp.*
			HP-112	*H. pylori /normal mucosa .*
681 A/-	NA	2	HP-11	*H. pylori gastritis.*
			HP-112	*H. pylori /normal mucosa .*

-NA: not identified.

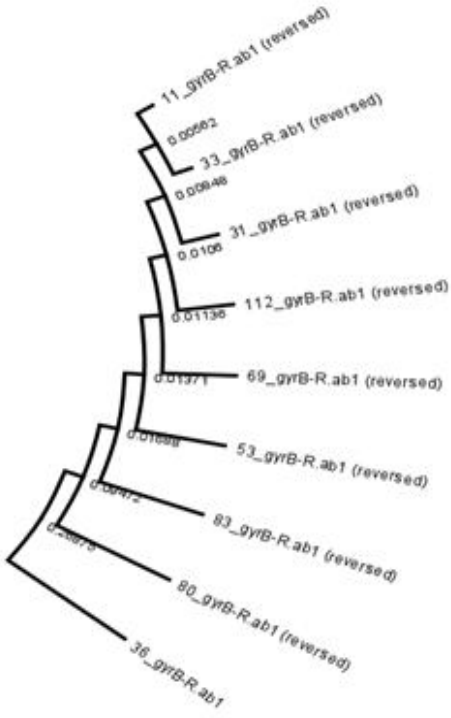
C. Analysis of partial sequencing tree of gyrA, gyrB and frxA.

Constriction of partial sequencing tree of *gyrA,gyrB* and *frxA* from 9 PCR amplified products related to 9 *H. pylori* strains identified in extracted DNA from GTBs of different 9 patients were showed in Fig.1a,b,c. Tree of partial sequencing of *gyrA,* revealed that there were two clades according to nucleotide changes, Fig. 1a. Clad-A include strains HP-36 and HP53 as sister taxa with genetic distance ($3.104\ X10^{-7}$) and strains HP-11 and HP-112 as sister taxa with genetic distance (0,00835). In contrast, strain HP-31 considered as out-group taxa for HP-36 and HP-53 with genetic distance 0.00184. Clad-B include strains HP-69 and HP-80 as sister taxa with genetic distance (0.0074). Strains HP-83 and HP-33 considered as out-group taxa for HP-69 and HP-80 with genetic distance 0.01117 and 0.01151, respectively.

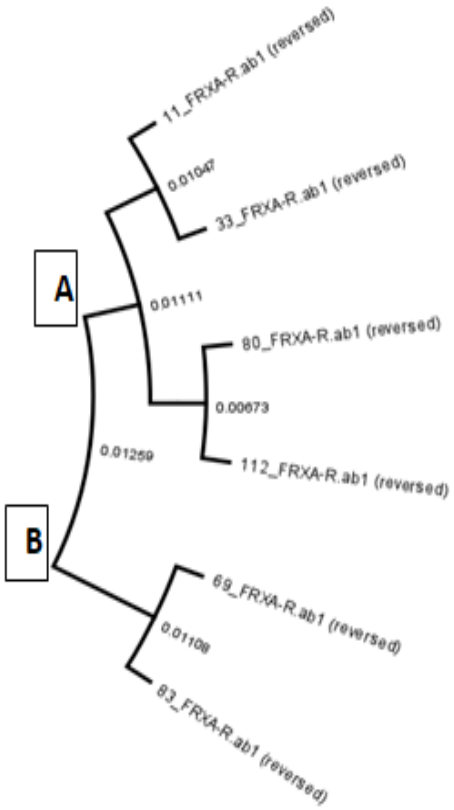
Partial sequencing tree of *gyrB* revealed that strains HP-11 and HP-33 were considered as sister taxa with genetic distance 0.0056 , and strains HP-31,HP-112,HP-69,HP-53,HP-83,HP-80 and HP-36 considered as out-groups taxa for HP-11 and HP-33 with genetic distance 0.00848 , 0.0106 , 0.01136 , 0.01371, 0.01688 , 0.09472 , 0.26876, respectively, Fig.1b. Tree of partial sequencing of *frxA* revealed that there were two clades according to nucleotides changes Fig.1c. Clad-A include strains HP-11 and HP-33 as sister taxa with genetic distance (0.01047) and strains HP-80 and HP-112 as sister taxa with genetic distance (0.00673). Genetic distance between these two sisters taxa was 0.01111. Clad-B included strains HP-69 and HP-83 as sister taxa with genetic distance (0.01108). Genetic distance between these two clads was (0.01259).


36_gyrA-R.ab1 (reversed)
3.104x10⁻⁴
53_gyrA-R.ab1 (reversed)
0.00184
31_gyrA-R.ab1 (reversed)
0.00915
A
11_gyrA-R.ab1 (reversed)
0.00835
112_gyrA-R.ab1 (reversed)
0.0121
69_gyrA-R.ab1 (reversed)
0.0074
B
80_gyrA-R.ab1 (reversed)
0.01117
0.01151
83_gyrA-R.ab1 (reversed)
33_gyrA-R.ab1 (reversed)

(a)


11_gyrB-R.ab1 (reversed)
0.00562
33_gyrB-R.ab1 (reversed)
0.00948
31_gyrB-R.ab1 (reversed)
0.0106
112_gyrB-R.ab1 (reversed)
0.01136
69_gyrB-R.ab1 (reversed)
53_gyrB-R.ab1 (reversed)
83_gyrB-R.ab1 (reversed)
80_gyrB-R.ab1 (reversed)
36_gyrB-R.ab1

(b)



(c)

Fig. 1. (a): Partial Sequencing Tree Of gyrA. The Analysis Involved 9 Strains Of H. Pylori In Comparison With Standard Strains (SJM180, HP42K, BGD133, ATCC 49503, BGD 133, 26-A-EK1, PUNO-003, B125A, And BGD54) From The NCBI Gene Bank Database. All Positions Containing Gaps And Missing Data Were Eliminated. The Number On The Branches Referred To Genetic Distance Between Sub-Clusters. (B): Partial Sequencing Tree Of Gyrb. The Analysis Involved 9 Strains Of H. Pylori In Comparison With Standard Strains (C-Mx-2011-171, CC33C , RIGLD OC 250 , Aklavik86 , BGD52 , BGD109 , MT5105 , AL05 , C-Mx-2011-145) From The NCBI Gene Bank Database. All Positions Containing Gaps And Missing Data Were Eliminated. The Number On The Branches Referred To Genetic Distance Between Sub-Clusters. (C): Partial Sequencing Tree Of Frxa .The Analysis Involved 6 Strains Of H. Pylori In Comparison With Standard Strains(NCTC 12823, B140 , C-Mx-2010-8 , OKI102 , NCTC 12823)From The NCBI Gene Bank Database. All Positions Containing Gaps And Missing Data Were Eliminated. The Number On The Branches Referred To Genetic Distance Between Sub-Clusters.

DISCUSSION

In this study, several nucleotides change where detected using partial sequencing of *gyrA*, *gyrB* related with levofloxacin resistance and *frxA* related with metrandizol resistance. Some of synonymous and non- synonymous nucleotide changes vary among studied samples by their locations and types. For *gyr*A and *gyr*B, the frequency of nucleotide changes was higher in *gyrA*, especially in strain HP-11 which harbor 10 nucleotide changes (9 synonymous and 1 non-synonymous) followed by strain HP-112 with 9 nucleotide changes (8 synonymous and 1 non-synonymous). For *gyrB,* the higher frequency of nucleotide changes was in strain HP-83 with 8 nucleotide changes all were synonymous followed by strains HP-33,HP-53,and HP-112 which all have 7 nucleotide changes.

Nucleotide changes in *gyrA* is more frequency than *gyrB* because resistance to levofloxacin is primarily mediated through point mutations in *gyrA*[12],[13]. As for one of metrondazole resistance gene *frxA,* the frequency of

nucleotide changes was highest in strain HP-69 with 14 nucleotide change, all are non-synonymous. Clinical presentation of patient from who strain HP-69 was identified, was gastric adenocarcinoma cancer. Strains HP-11 and HP-33 showed (7 synonymous and 4 non-synonymous) nucleotide changes, even though the clinical presentations of patients from who strains HP-11 and HP-33 was identified, were gastritis and normal mucosa, respectively. The presence or absence of mutation does not effect the progress of the disease but may effect the speed of disease development, that may be because *H. pylori* exhibits unusual genetic flexibility and it was hypothesized that the variability within the genome could potentially account for the organism's ability to adapt the dynamic environment within the host gastric niche, facilitating chronic colonization[14].

In the current study, a high frequency of nucleotide changes in *frxA*, that may be because *frx*A is the second nitro-reductase protein in *H. pylori* .Nitro-reductases (NTR) are a family of proteins involved in the reduction of nitro-containing compounds. The role of nitro-reductases is to activate the antimicrobial by reducing the nitro group. A decrease in the activity of nitro-reductases associated with antibiotic resistance [15]. Also, inactivation of *frx*A leads to reduction in transformation (recovery) of metronidazole into the active derivatives (NO2- and NO2 2-), that have a damaging effect on the structure of DNA of *H. pylori*[16].

In the current study, analysis of partial sequencing of *gyr*A,*gyr*B and *frx*A in studied *H. pylori* strains in comparison with nucleated sequences of different stander strains showed that several nucleotides change are common between the studied strains, even the comparison was done with different stander strain. For *gyr A*, the frequent nucleotide change was (C/T) at position 58 which identified in strains HP-11,HP-31,HP-83 and HP-112 ,and (T/C) at position 392which identified in strains HP-11, HP-83 and HP-112. Most of these nucleated changes were un-conformed by previous studies if they are related to antibiotic resistance or not. Nucleotide change (C/T) at position 130 which identified in strains HP-36 and HP-53 was confirmed by previous study to be relate to antibiotic resistance of levofloxacin[17]. For *gyr B,* the most frequent nucleotide change was (A/G) at position 445which identified in strains HP-11, HP-31, HP-33, HP-69, HP-80, HP-83and H-112. All of the detected nucleotide changes in *gyrB* were unconfirmed in previous studies as have relation with resistance to levofloxacin. For *frxA,* most frequent nucleotide change was (T/A) at position 22 in strains HP-11 and HP-112. None of the nucleotide changes in *frxA* were confirmed by previous studies to be related to antibiotic resistance. This indicate that *H pylori* has a remarkable genetic variability and high allelic diversity, determining that strains from different patients may have distinct genotypes; this driven by an elevated mutation rate and frequent intraspecific recombination that contribute to host adaptation by the increment of antibiotic resistance probabilities[18].The presence of frequent nucleotide changes in the genes is mainly because of a certain DNA repair systems, such as components of the mismatch repair system, which is absent in the *Helicobacter* species. Persistent accumulation of mutations within the genome may make an important contribution to the extraordinary genetic diversity of *H. pylori* and allow adaptation to new environmental challenges within the stomach [19].

In the current study, the association between presence of non-synonymous mutation in *gyrA,gyrB and frxA*, and clinical presentations of patients from who the strains was identified was statically not significant. That may be due to the limitation of studied samples number [20]. Reactive Oxygen Species (ROS) are produced by many host cells such as neutrophils and epithelial cells in response to *H. pylor* ,this leads to chronic infection causing inflammation, oxidative stress, and damage to gastric mucosa eventually resulting in gastric carcinoma[21].

In the current study, trees of partial sequencing of *gyrA* , *gyrB* and *frxA* give more clear association between the presence of nucleotide changes and the histo-pathological and clinical presentation of patients from who *H. pylori* strains were identified. From tree of partial sequencing of *gyr*A , HP-36 and HP-53 strains consider as sister taxa with genetic distance of ($3.104x10^{-7}$) and the clinical presentation of patients from who the strains HP-36 and HP-53were identified were (*H pylori* gastritis/gastric polype ,ulceration) and (*H. pylori* /normal mucosa),respectively, as both of the those patients have *H. pylori* infections but one patient with more advance stage of the disease and the second patient with normal mucosa even these two strains of *H. pylori* were genetically so close. That mean the sister taxa have low genetic divergence between them and the second patient(who displayed normal mucosa) may be in early stage of the disease and have higher chance to develop

advance degree of disease. Another example was strain HP-69 (which identified from patient with gastric adenocarcinoma) and strain HP-80 (which identified from patient with *H. pylori* active chronic gastritis, intestinal metaplasia, focal regenerative surface, glandular epithelial hyperplasia but without malignancy).Genomic distance between these two strains was (0.0074) and they are sister taxa.That means the second patient may be, as soon as far, will progress to malignancy.

From tree of partial sequencing of *gyr B*, strain HP-11 (which identified from patient with *H. pylori* gastritis) and strain HP-53 (which identified from patient with *H. pylori* /normal mucosa), were considered as sister taxa with genetic distance 0.0056. The second patient may be in early stage of the disease and have higher chance to develop advance degree of disease.

From tree of partial sequencing of *frx A*, strain HP-11 (which identified from patient with *H. pylori* gastritis) and strain HP-33 (which identified from patient with *H. pylori* /normal mucosa) were sister taxa with genetic distance (0.01047). The second patient may be in early stage of the disease and have higher chance to develop advance degree of disease. Another example, strain HP-69 (which identified from patient with *H. pylori* /gastric adenocarcinoma) and strain HP-83(which identified from patient with *H. pylori* /gastric polyp) , were sister taxa with genetic distance (0.01108). There was association between the disease development and the genetic distance between the two strains because gastric adenocarcinoma is cancer type and gastric polyps are intraluminal projections of mucosal or sub-mucosal tissue. They are generally asymptomatic and, as such, are typically found incidentally on upper endoscopy. While these lesions are typically benign, they do have the potential of containing local dysplasia and progression to invasive cancer [22].

H. pylori genotypic resistance testing might be a good alternative to phenotypic resistance testing. Some genetic mutation loci have been reported to contribute to the *H. pylori* phenotype, and phenotypic resistance tests for clarithromycin and quinolones have been partially replaced by genotypic resistance tests. However, the loci of *H. pylori* conferring resistance to other antibiotics are controversial, and further studies are needed to determine which genetic mutations are responsible for resistance[23].

CONCLUSION

Applications of direct molecular identification of bacteria and susceptibility to the most important antibiotics used in treatment of *H. pylori* as routine test in bacteriology labs are most important.

CONFLICT OF INTEREST

There is no conflict of interest

FUNDING

Authors funding the work.

AUTHOR CONTRIBUTION

A.A.M. contributed to implementation of the research project and writing the draft of manuscript. M.A.R.D. contributed to the suggestion of the project idea, interpretation of analytical data, proofreading of research, and writing and producing the research in its final form.

REFERENCES

[1] L. Boyanova, P. Hadzhiyski, R. Gergova, and R. Markovska," Evolution of Helicobacter pylori Resistance to Antibiotics: A Topic of Increasing Concern", Antibiotics, vol. 12,no. 4,pp. 332,2023.

[2] Y. Zhang, X. Feng, L. Bian, Y. Zhang, Q. Li, Y. Xu, Q. She, C. Yan, G. Lu, J. Wu, W. Xiao, Y. Ding, and B. Deng,''Antibiotic Resistance of Helicobacter pylori and Related Risk Factors in Yangzhou, China: A Cross-Sectional Study'', Journal of clinical medicine, vol.12,no. 3, pp.816,2023.

[3] V. P. Tuan, D. Narith, E. Tshibangu-Kabamba, H. D. Q. Dung, P. T. Viet, S. Sokomoth, T. T. Binh, S. Sokhem, T. D.Tri, S. Ngov, P. H. Tung, N. P. M. Thuan, T. C. Truc, B. H. Phuc, T. Matsumoto, K. A. Fauzia, J. Akada, T. T. H. Trang, and Y.Yamaoka,'' A Next-Generation Sequencing-Based Approach to Identify Genetic Determinants of Antibiotic Resistance in Cambodian Helicobacter pylori Clinical Isolates'', Journal of clinical medicine, vol. 8,no.6,pp. 858,2019.

[4] K. A. Fauzia, H. Aftab, E. Tshibangu-Kabamba, R. I. Alfaray, B. Saruuljavkhlan, A. Cimuanga-Mukanya, T.Matsumoto, P. Subsomwong, J. Akada, M. Miftahussurur, and Y. Yamaoka, ''Mutations Related to Antibiotics Resistance in Helicobacter pylori Clinical Isolates from Bangladesh'', Antibiotics, vol. 12,no.2,pp. 279, 2023.

[5] S.Hu, Y. Zhou, Y. Deng, Y. Bo, X. Chen, W. Yang, R. Shi, W. Zhao, Z. Hou, J. Hu, J. Liu, X. Zhang, H. Yong, P. Wang, F. Li, H. Qi, X. Wang, L. Jin, T. Cui, H. Yong, H. Gao, ''Characteristics of phenotypic antibiotic resistance of Helicobacter pylori and its correlation with genotypic antibiotic resistance: A retrospective study in Ningxia'', Helicobacter, vol.28,no. 3,pp. e12960, 2023.

[6] A. Bińkowska, M. M. Biernat, Ł. Łaczmański, and G. Gościniak,''Molecular Patterns of Resistance Among Helicobacter pylori Strains in South-Western Poland'', Frontiers in microbiology, vol. 9, pp. 3154, 2018.

[7] Y. Zhang, Y. Wen, Q. Xiao, W. Zheng, G. Long, B. Chen, X. Shu, and M. Jiang,'' Mutations in the Antibiotic Target Genes Related to Clarithromycin, Metronidazole and Levofloxacin Resistance in Helicobacter pylori Strains from Children in China'' Infection and drug resistance, vol. 13,pp. 311–322,2020.

[8] I. M. Saracino, M. Pavoni,A. Zullo, G. Fiorini, T. Lazzarotto, C. Borghi, and D. Vaira, ''Next Generation Sequencing for the Prediction of the Antibiotic Resistance in Helicobacter pylori: A Literature Review''. Antibiotics ,vol. 10,no.(4),pp. 437,2021.

[9] S. I. Smith, K. S. Oyedeji, Arigbabu, A. O., Cantet, F., Megraud, F., Ojo, O. O., Uwaifo, A. O., Otegbayo, J. A., Ola, S. O., & Coker, A. O. (2004). Comparison of three PCR methods for detection of Helicobacter pylori DNA and detection of cagA gene in gastric biopsy specimens. World journal of gastroenterology, 10(13), 1958–1960.

[10] Akeel, M., Shehata, A., Elhafey, A., Elmakki, E., Aboshouk, T., Ageely, H., & Mahfouz, M. (2019). Helicobacter pylori vacA, cagA and iceA genotypes in dyspeptic patients from southwestern region, Saudi Arabia: distribution and association with clinical outcomes and histopathological changes. BMC gastroenterology, 19(1), 16.

[11] Mirzaei, N., Poursina, F., Moghim, S., Rahimi, E., & Safaei, H. G. (2014). The mutation of the rdxA gene in metronidazole-resistant Helicobacter pylori clinical isolates. Advanced biomedical research, 3, 90.

[12] L. H. Wang, H. Cheng, F. L. Hu, and J. Li,''Distribution of gyrA mutations in fluoroquinolone-resistant Helicobacter pylori strains'' World journal of gastroenterology, vol.16, no.18,pp. 2272–2277,2010.

[13] S. Y. Rhie, J. Y. Park, T. S. Shin, J. W. Kim, B. J. Kim, and J. G. Kim,''Discovery of a Novel Mutation in DNA Gyrase and Changes in the Fluoroquinolone Resistance of Helicobacter pylori over a 14-Year Period: A Single Center Study in Korea'' Antibiotics, vol. 9, no. 6), pp. 287, 2020.

[14] K. P.Haley, and J. A. Gaddy,''Helicobacter pylori: Genomic Insight into the Host-Pathogen Interaction'', International journal of genomics, pp. 386905,2015.

[15] C. Thomas, and C.D. Gwenin,''The Role of Nitroreductases in Resistance to Nitroimidazoles'', Biology, vol. 10,no. 5, pp. 388, pp. 2021.

[16] E. G. Chua, A. W. Debowski, K. M. Webberley, F. Peters, B. Lamichhane, M. F. Loke, J. Vadivelu, C. Y. Tay, , B. J. Marshall, and M. J. Wise, ''Analysis of core protein clusters identifies candidate variable sites conferring metronidazole resistance in Helicobacter pylori'', Gastroenterology report, vol.7, no.1, pp. 42–49, 2019.

[17] M. Miftahussurur,P.K. Shrestha, P. Subsomwong, R.P. Sharma and Y. Yamaoka'' Emerging Helicobacter pylori levofloxacin resistance and novel genetic mutation in Nepal'', BMC Microbiol, vol. 16, pp. 256, 2016.

[18] M. López-Gasca, J. Peña, M. A. García-Amado, F. Michelangeli, and M. Contreras,''Point Mutations at gyrA and gyrB Genes of Levofloxacin-Resistant Helicobacter pylori Isolates in the Esophageal Mucosa from a Venezuelan Population'', The American journal of tropical medicine and hygiene, vol.98, no. 4,pp. 1051–1055, 2018.

[19] A. Labigne, and P.J. Jenks,''Mutagenesis, Helicobacter pylori: Physiology and Genetics. HLT Mobley, GL Mendz ,SL Hazell SL (Eds.). ASM Press. Washington (DC).USA . Chapter 30.

[20] H. Y. Kim,''Statistical notes for clinical researchers: Sample size calculation 2. Comparison of two independent proportions'', Restorative dentistry & endodontics, vol. 41, no. 2,pp. 154–156, 2016.

[21] Y. S. Cho, S. Y. Nam, H. S. Moon, T. H. Kim, S. E. Kim, and J. T. Jung,''Helicobacter pylori eradication reduces risk for recurrence of gastric hyperplastic polyp after endoscopic resection''. The Korean journal of internal medicine, vol. 38, no. 2,pp.167–175, 2023.

[22] R. Castro , P. Pimentel-Nunes, and M. Dinis-Ribeiro. ''Evaluation and management of gastric epithelial polyps '', vol.31, no. 4, pp.381-387, 2017. https://doi.org/10.1016/j.bpg.2017.06.001.

[23] Y. Zhou, Z. Zhong, S. Hu, J. Wang, Y. Deng, X. Li, X. Chen, X. Li,Y. Tang, X. Li, Q. Hao, J. Liu, T. Sang, Y. Bo, and F. Bai, ''A Survey of Helicobacter pylori Antibiotic-Resistant Genotypes and Strain Lineages by Whole-Genome Sequencing in China'', Antimicrobial agents and chemotherapy, vol. 66, no. 6, pp.e0218821,2022.