Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.47750/jptcp.2023.30.04.018

Molecular Detection of Antimicrobial Resistant Genes to Clarithromycin in Helicobacter pylori at Basrah, Iraq

Raneem A. Kareem[1], Lamyaa kadhim baqer[2], hayder mohsin jarullah[3]
[1,2] Department of Microbiology, College of Medicine, University of Basrah, Basrah, Iraq
[3] Iraqi Ministry of Health, gastroenterology and hepatology hospital, Basrah, Iraq
***Corresponding author:** Raneem A. Kareem, Department of Microbiology, College of Medicine, University of Basrah, Basrah, Iraq, Email : raneem.abdulkareem@bjes.edu.iq

Submitted: 12 January 2023; Accepted: 15 February 2023; Published: 13 March 2023

ABSTRACT

The primary cause of gastritis and peptic ulcers, as well as gastric cancer and gastric mucosa-associated lymphoid tissue lymphoma, is the bacterium Helicobacter pylori. When an infection with H. pylori is discovered, eradication therapy should be started, and it is best if it is successful the first time. According to international standards, in some instances, medication susceptibility testing should be used to tailor treatment. The most widely used first-line treatment is still triple therapy, which combines a proton-pump inhibitor (PPI) with amoxicillin and clarithromycin (PPI-AC). This case-control study included 112 patients (55 males and 57 females), aged between 15 and 74, with a variety of gastritis symptoms, and 112 randomly chosen controls (59 males and 53 females), aged between 15 and 74, who were H. pylori negative. RUT and PCR were used for diagnosis of the bacteria. Real-time PCR was used to genotype two SNPs of the gene 23S rRNA, A2142G, and A2143G, which confer clarithromycin resistance. The rate of resistance to clarithromycin were 65.2% which consider high according to similar studies and this resistant appear that it was not associated with any of age, diabetes and smoking and it was result from point mutation in 23sRNA gene.

Keywords: *H. pylori, 23sRNA, Antibiotic resistant genes*

J Popul Ther Clin Pharmacol Vol 30(4):e181–e194; 13 March 2023.

INTRODUCTION

Helicobacter pylori (H. pylori) is a spiral-shaped, flagellated, microaerophilic, extracellular bacteria that inhabits the human gastric sub-mucosa. Recent research has revealed that H. pylori was prevalent in the early east African progenitor population a long time ago, despite the fact that this microbe was first introduced in Australia by Barry Marshall and Robin Warren in 1982 [1]. Gastric adenocarcinoma, peptic ulcer, chronic gastritis, and mucosa-associated lymphoid tissue (MALT) lymphoma are all primarily brought on by Helicobacter pylori [2]. H. pylori infected approximately 4.4 billion people worldwide. Africa (79.1%), Latin America (63.4%), and Asia (54.7%) reported the greatest incidence rates of infection, whereas Northern America (37.1%) and Australia (24.4%) recorded the lowest prevalence rates [1].

The high water pollution in Basra province saw in the middle of 2018 was an upsurge in H. pylori infection, at various ages, the infection rate was 58%, and more males than women were affected [3].

The most frequently prescribed first-line therapy is still triple therapy, which combines a proton-pump inhibitor (PPI) with amoxicillin and clarithromycin (PPI-AC). This combination replaced the less successful triple therapies as the initial therapy that was highly recommended. Over time, it has received highly favorable evaluations. Pretreatment clarithromycin resistance is a key factor in the efficacy of this combination in eradicating the disease (CR) [4], clarithromycin, amoxicillin, levofloxacin, metronidazole, tetracycline, rifabutin, and substances containing bismuth are all included in the H. pylori therapy program [5].

After 14 days, the eradication rate of PAC was higher than it was after 7 days (81.9% vs. 72.9%). In another study, we looked at the rate of PAC eradication [6]. A meta-analysis of 3715 patients in Turkey revealed that the eradication rate was incredibly low (60%) regardless of whether the therapy lasted for 7 days or 14 days, which may be related to the high level of clarithromycin resistance in the area [7].

When treating H. pylori infections, there is a serious concern about the growing resistance to first-line treatments. Clarithromycin, levofloxacin, metronidazole, amoxicillin and tetracycline, are the most frequently used antibiotics in the treatment of H. pylori. However, the effectiveness of the majority of these medicines has dramatically decreased due to the developing antimicrobial resistance (AMR) rate in H. pylori, which has caused the eradication rates to decline to an unsatisfactory level [8]. Numerous PCR-based studies have demonstrated that point mutations in the peptidyl transferase loop of the 23S rRNA gene's V domain are the root cause of the CLA resistance phenotype in clinical H. pylori strains from various geographical locations. These alterations have the capacity to alter the shape of the peptidyl transferase loop and hinder CLA's ability to bind to the 23S rRNA, reducing its effectiveness and producing a resistant phenotype. The most common and well-documented mutations in H. pylori are adenine-to-guanine transitions at positions 2142 (A2142G) or 2143 (A2143G) or, less frequently, an adenine-to-cytosine transversion at position 2142 (A2142C), which together account for more than 90% of CLA resistance in developed nations [9]. It has been observed that other mutations, such as A2115G, G2141A, A2144T, and T2289C, can confer CLA resistance [10].

MATERIAL AND METHODS

Gastric biopsy samples collection

This study is a case-control study A total of 112 patients (55 males and 57 females) with age group from (15-74) with various gastritis symptoms attending endoscopy unit at Hospital of gastroenterology and hepatology in Basra during period from (1th November 2021 to 15th of February 2022) were underwent endoscopic examination. A gastroenterologist performed the endoscopic examinations and recorded the results.

All the patients had the main symptoms and signs like epigastric pain, bloating, vomiting and nausea. A questionnaire was filled by direct interview with each patient. It covers questions related to epidemiological, clinical features and laboratory investigations of the patients.

There were112 controls (59 males and 53 females), with age groups from (15-74) years, whom H. pylori negative, were selected randomly.

All patients who have autoimmune diseases, who have been treated with antibiotic or Proton Pump Inhibitors (PPI) or had discontinued previous

treatment and History of previous gastric surgery where excluded from the study.

Three gastric biopsy specimens from the antrum and lesser curvature were obtained from each patient for determining H. pylori Infection, two biopsy was used for detection of H. pylori by rapid urease test and one biopsy was used for conventional PCR to diagnosis the bacteria and to detect mutation in 23s RNA by real time PCR.

Ethical approval

The ethical approval was under acceptance by the ethical approval committee in the College of Medicine, the Basrah University offer acceptance and approval of research and development center, the Ministry of Health.

Biochemical Test

Rapid Urease Test (RUT)

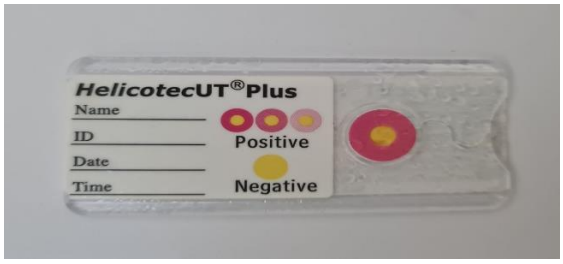
The H. pylori urease enzyme is detected by this assay in the stomach biopsy.

Reading the results

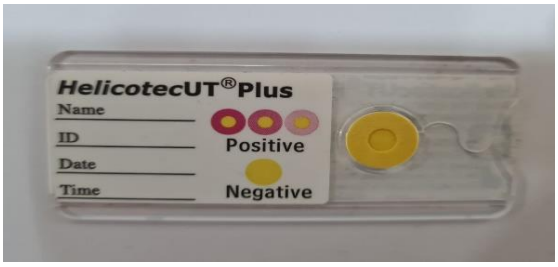
After few seconds (Fig 1):

A: + ve result ---- the solution color changed to pink

B: – ve result ---- no change in the color



A: Positive result



B: negative result

Bacterial DNA extraction

DNA extraction according to manufactiured company (Presto™ Mini gDNA tissue Kit, Geneaid, China).

Molecular detection of H. pylori by conventional PCR

GoTaq® G2 Green Master Mix is a high-quality Taq DNA Polymerase, deoxynucleotides, and reaction buffer solution prepared for use in a 2X concentration. It includes all of the tools required for DNA amplification. The primer pairs were designed to detect gyr A gene which is consider as a housekeeping gene (table 1). Preparation of conventional PCR solution and Thermal Cycler Programs Used in this study were demonstrated in table (2), (3).

TABLE 1: Primers that used in this study

Gene	Primer name	5'-3'	Product size (bp)	Accession number	Reference	Application
gyrA	gyrAHP-1F	AGCTTATTCCATGAGCGTGA	581	CP051541	Wang(2010)	Conventional PCR
	gyrAHP-1R	TCAGGCCCTTTGACAAATTC				

TABLE 2: Preparation of conventional PCR solutions

Components	Concentration	Volume (50 µl)
2X PCR Taq Master Mix	1X	25 µl
Forward primer	10 µM/µl	4 µl
Reverse primer	10 µM/µl	4 µl

0ddH2O	-	13 µl
DNA	40 ng	4 µl

TABLE 3: Conventional PCR conditions

Phase	Ta (°C)	Time	Cycles
Initial denaturation	94°C	5 min	1X
Denaturation	94°C	30 sec.	35X
Annealing	54°C	30 sec.	
Extension	72°C	1 min	
Final extension	72°C	5 min	1X

DNA Sequencing

All isolates of H. pylori for the gene gyr A were selected for sequencing were sent to macrogen laboratories in Korea to confirm H. pylori isolates.

Real-Time PCR assay (for SNP detection of gene 23S rRNA)

All of the suspicious samples were used in the traditional Real-Time PCR process. The conserved portion of Helicobacter pylori's 23S rRNA gene was amplified using a single set of specialized primers. Using primers and probes designed specifically for these two SNPs, genotypes of the 23S rRNA gene's two A2142G and A2143G SNPs (Table 4) were determined. 2 µl of genomic DNA, a GoTaq® Probe qPCR Master Mix were conducted in 0.2 wells in a total volume of 20 µl that were used in the reactions (Promega, USA). Real-time PCR settings and solution preparation are provided in Tables (5) and (6).

TABLE 4: real time PCR primer and probes

Gene	Primer name	5'-3'	Product size (bp)	Accession number	Reference	Application
23S rRNA	23SHP-RTF	GAGCTGTCTCAACCAGAG	127	NM_002046.7	Modified from Gonzalez-Hormazabal et al., 2018	RT-qPCR assay
	23SHP-RTR	GCGCATGATATTCCCATTA				
	23SHP-G42	HEX-5'-CAAGACGGGAAGACCCC-3'-BHQ1				
	23SHP-G43	Cy3-5-CAAGACGGAGAGACCCC-3'-BHQ2				
	23SHP-AA	FAM-5'-CAAGACGGAAAGACCCCG-3'-BHQ1				

TABLE 5: Real-Time PCR conditions

Phase	Tm (°C)	Time	Cycles
Initial denaturation	95°C	10 min	1X
Denaturation	95°C	15 sec.	40X
Annealing	60°C	1 min	
Final Extension	72°C	30 sec	

TABLE 6: Preparation of Real-Time PCR solutions

Components	Concentration	Volume (20 µl)
ddH2O	-	3 µl
GoTaq® Probe qPCR Master Mix	1X	10 µl
Forward primer	10 µM/µl	1 µl
Reverse primer	10 µM/µl	1 µl
Probe 1 (FAM)	10 µM/µl	1 µl
Probe 2 (HEX)	10 µM/µl	1 µl
Probe 3 (Cy3)	10 µM/µl	1 µl
DNA	40 ng	2 µl

Statistical analysis

Data were fed into SPSS, version 24 for tabulation and analysis of data, p- value was used for comparison between the data.

RESULT

DNA amplification

DNA extracted from Biopsy have been amplified by using conventional PCR, then PCR product results confirmed by using the gel was 1.5% and the DNA dye is RedSafe (Intron, Korea). V: 90, Time: 45 minutes. M: Ladder in this analysis the DNA band that appear on the gel after successful attachment between DNA template that had been extracted and the goal specialized primer for gyr A as seen in figure 2 , the bands appeared under UV imaging system as orange compact bands due to the DNA staining that used as indicator which was RedSafe stain, the bands of extracted DNA can be estimated on gel electrophoresis by using DNA band size indicator that was (100- 1500) DNA ladder, each Gene can be revealed on the results of amplified DNA, illustrated in figure (2).

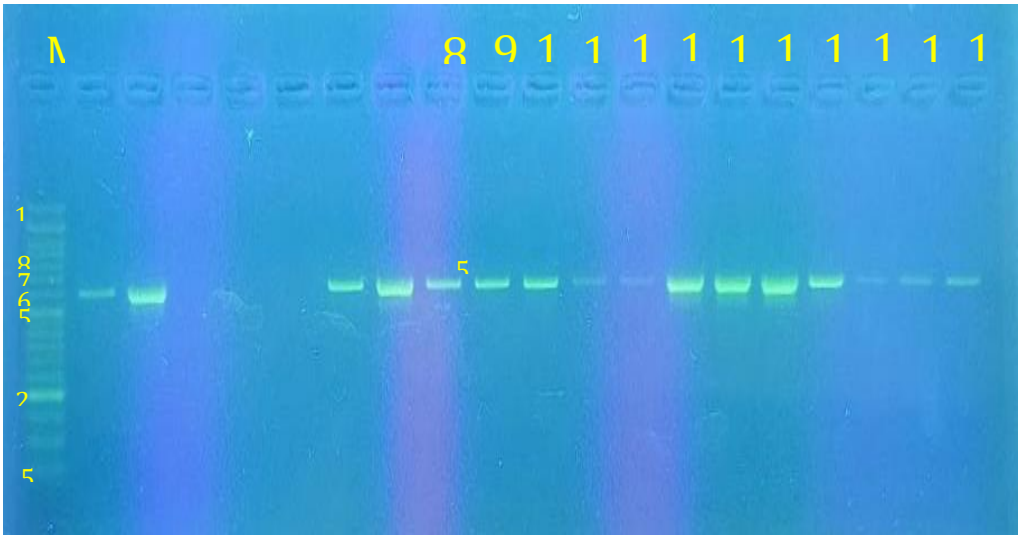


FIGURE 2: show PCR products of the detection of gene gyrA of Helicobacter pylori. The size of the PCR product for gyrA is 581 bp. Ta= 54 ˚C. The gel was 1.5% and the DNA dye is RedSafe (Intron, Korea). V: 90, Time: 45 minutes. M: DNA ladder

Rapid urease test result and its relation to PCR result

Table (7) shows the relation between Rapid urease test and PCR which appear with 94.6% sensitivity and 97.3 % specificity.

TABLE 7: The relation between Rapid urease test and PCR

Rapid urease test	PCR		Total
	Positive	Negative	
Positive	106	3	109
	94.6%	2.7%	48.6%
Negative	6	109	115
	5.3%	97.3%	51.3%
Total	112	112	224
	100.0%	100.0%	100.0%

* Chi-Square Test

Sensitivity= 94.6%
Specificity= 97.3%

Phylogenetic tree of H. pylori

Forward and reverse primer isolate phylogenetic analyzes were analyzed using Neighbour-Joining method and compared with the different H. pylori sequences available in the Gen Bank database, there is no convergence between our H. pylori isolates and these of Gen Bank, as seen in the figure (3).

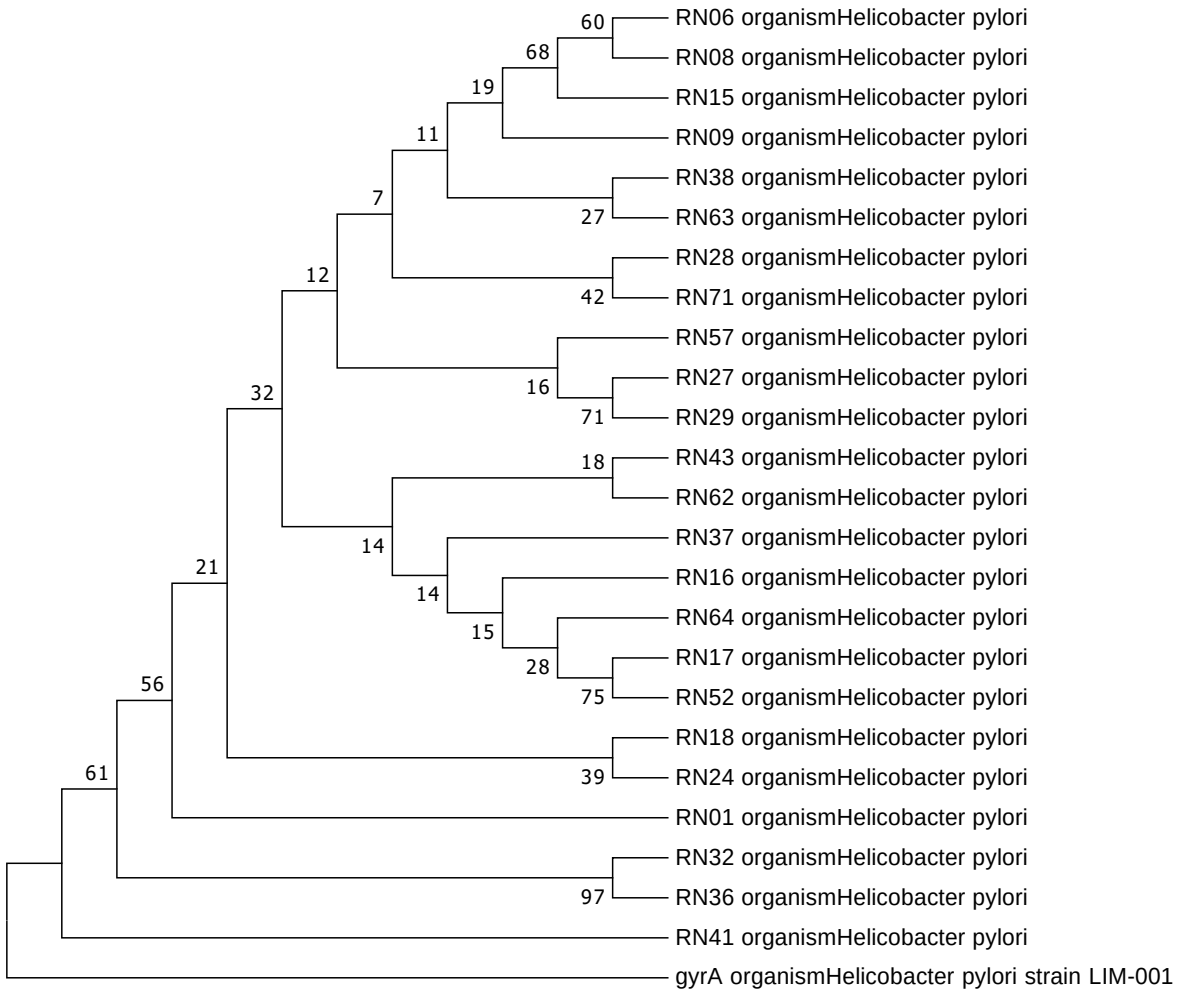


FIGURE 3: Evolutionary relationships of partial sequences of gene gyrA in the isolates of Helicobacter pylori strains isolated from Iraqi patients.

Amplification plot of RT-PCR for detection of two SNPs in gene 23S rRNA of Helicobacter pylori

The conserved portion of Helicobacter pylori's 23S rRNA gene was amplified using a single set of specialized primers. Two SNPs of gene 23S rRNA, A2142G and A2143G, as shown in figures (4), specific SNPs were genotyped using primers and probes.

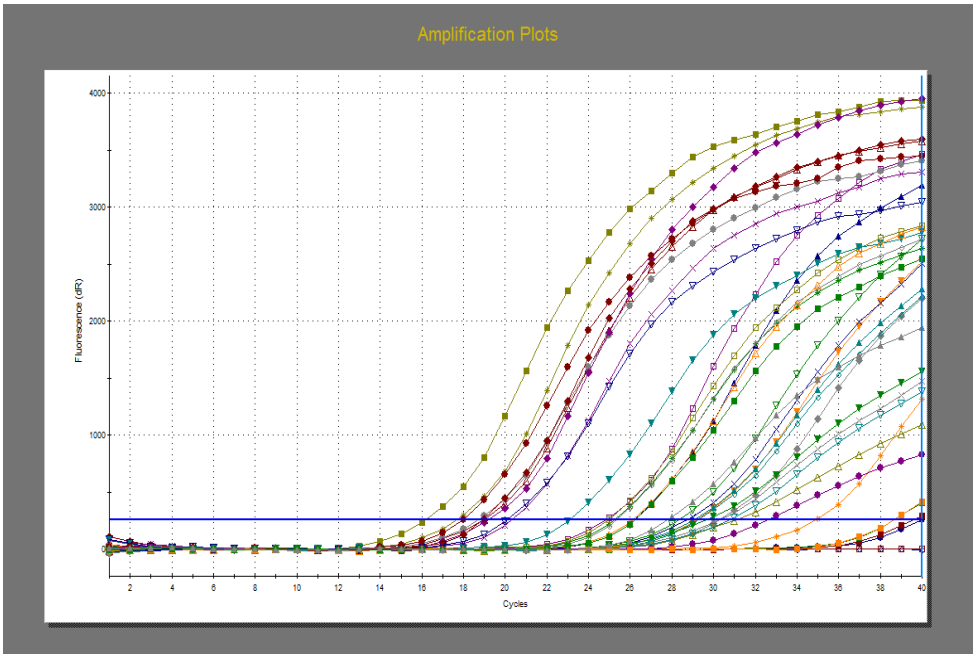


FIGURE 4: Amplification plot of RT-PCR for detection of two SNPs in gene 23S rRNA of Helicobacter pylori.

Distribution of clarithromycin resistant and sensitive strain

Figure (5) shows the percentage of clarithromycin resistant and sensitive strain which appear as follows:

Resistant	65.20%
Sensitive	34.80%

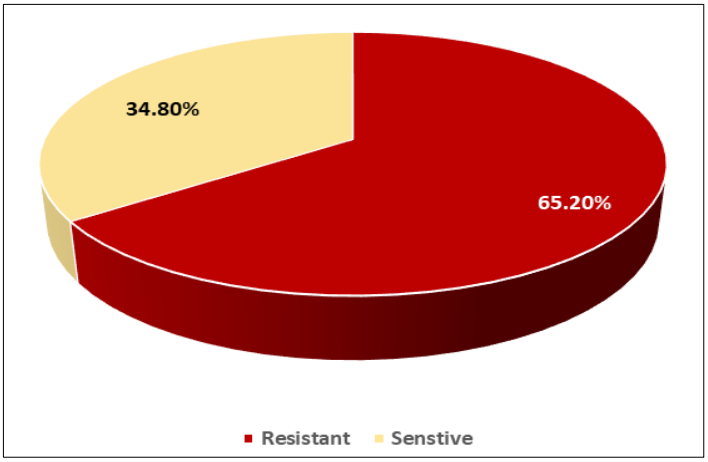


FIGURE 5: percentage of resistant and sensitive strain

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Distribution of clarithromycin resistant strain according to Age

Table (8) Shows the distribution of clarithromycin resistant strain according to age which appear that the most number of resistant strain 29 (39.7%) were found in age group (26-35) statistically this differences were non-significant p-value (0.140).

TABLE 8: distribution of clarithromycin resistant strain according to age

Age		Clarithromycin		Total	P- Value
		Resistant	Sensitive		
15-25	Count	15	7	22	0.140
	%	20.5%	17.9%	19.6%	
26-35	Count	29	7	36	
	%	39.7%	17.9%	32.1%	
36-45	Count	8	10	18	
	%	11.0%	25.6%	16.1%	
46-55	Count	9	6	15	
	%	12.3%	15.4%	13.4%	
56-65	Count	4	4	8	
	%	5.5%	10.3%	7.1%	
66-75	Count	8	5	13	
	%	11.0%	12.8%	11.6%	
Total	Count	73	39	112	
	%	100.0%	100.0%	100.0%	

* Chi-Square

Distribution of clarithromycin resistant strain according to smoking status

Table (9) Shows the distribution of clarithromycin resistant strain according to smoking which appear that the most number of resistant strain were found in non-smoker 50 (68.5%) statistically this differences were non-significant p-value (0.638).

TABLE 9: distribution of clarithromycin resistant strain according to smoking

Smoking			Clarithromycin		Total	P -Value
			Resistant	Sensitive		
Smoker	Yes	Count	23	14	37	0.638
		%	31.5%	35.9%	33.0%	
	No	Count	50	25	75	
		%	68.5%	64.1%	67.0%	
Total		Count	73	39	112	
		%	100.0%	100.0%	100.0%	

* Chi-Square

Distribution of clarithromycin resistant strain according to Diabetes status

Table (10) Shows the distribution of clarithromycin resistant strain according to diabetes status which appear that the most number of resistant strain were found in non-diabetes 45 (61.6%) statistically this differences were non-significant p-value (0.283).

TABLE 10: distribution of clarithromycin resistant strain according to diabetes status

Diabetes			Clarithromycin		Total	P – Value
			Resistant	Sensitive		
	Yes	Count	28	11	39	0.283
		%	38.4%	28.2%	34.8%	
	No	Count	45	28	73	
		%	61.6%	71.8%	65.2%	
Total		Count	73	39	112	
		%	100.0%	100.0%	100.0%	

* Chi-Square

Distribution of clarithromycin resistant strain according to clinical diagnosis

Table (11) Shows the distribution of clarithromycin resistant strain according to clinical diagnosis which appear that in patients with mild gastropathy there were 30 (41.1 %) of resistant strain whereas in patients with severe gastropathy there were 27 (37.0%) of resistant strain while the less number of resistant strain were found in patient with peptic ulcer, statically this differences were non-significant p-value (0.299).

TABLE 11: distribution of clarithromycin resistant strain according to clinical diagnosis

Diagnosis			Clarithromycin		Total	P-Value
			Resistant	Sensitive		
Diagnosis	Mild gastropathy	Count	30	22	52	0.299
		%	41.1%	56.4%	46.4%	
	Severe gastropathy	Count	27	11	38	
		%	37.0%	28.2%	33.9%	
	Peptic ulcer	Count	16	6	22	
		%	21.9%	15.4%	19.6%	
Total		Count	73	39	112	
		%	100.0%	100.0%	100.0%	

* Chi-Square

Distribution of point mutation in 23s RNA gene

Table (12) illustrated the distribution of point mutation in 23s RNA gene, it seen that the type of mutation A2142G was found more than A2143G in H. pylori strain.

TABLE 12: Illustrated the distribution of point mutation in 23s RNA gene

Gene		A2142G	A2143G	A2142G & A2143G	Total
23s RNA	Count	46	18	9	73
	%	63.0%	24.6%	12.3%	100%

DISCUSSION

According to the World Gastroenterology Organization's global guidelines, Helicobacter pylori (H. pylori) is present in half of the world's population and is the main culprit behind gastric carcinogenesis, along with chronic gastritis, gastroduodenal ulcers, and gastric mucosa-associated lymphoid tissue lymphoma [12].

The ACG guideline 2017 provides North American healthcare professionals with evidence-based, frontline treatment recommendations. These include levofloxacin triple therapy, concurrent therapy, sequential therapy, hybrid therapy, bismuth quadruple therapy, and clarithromycin triple therapy. A PPI, clarithromycin, and amoxicillin are all included

in the clarithromycin triple therapy (metronidazole if the patient is allergic to amoxicillin). The recommendation states that the treatment should last for 14 days when utilized in North America [13].

To successfully treat any bacterial infection, it is essential to identify germs that are resistant to antibiotics. This is especially true of H. pylori, which affects a large portion of a nation's population. The ability to rationally prescribe a treatment plan for patients is made possible by monitoring the background patterns of H. pylori's susceptibility to antibiotics. However, this baseline pattern of antibiotic susceptibility needs to be regularly tracked and adjusted throughout time.

The rise of H. pylori strains that are resistant to antibiotics has become a major problem worldwide, and numerous published studies have shown that both food-borne and clinical H. pylori strains exhibit high levels of antimicrobial drug resistance [14-17].

A total of 112 patients with various symptoms attributed to stomach and duodenum were subjected to endoscopy unit at the hospital of gastroenterology and hepatology.

By using the biopsy quick urease test and the polymerase chain reaction, the presence of an association with H. pylori was identified. Patients were regarded as having the infection if both test result was positive.

Rapid urease test result and its relation to PCR result

Due to the possibility of false negative results from several H. pylori infection diagnostic tests, using a variety of tests may aid in producing a more precise diagnosis [18,19].

The biopsy urease test in the endoscopic unit will be precise and useful, according to the study. The goal of this test performed during an endoscopy is to detect the presence of H. pylori, preventing patients from receiving unneeded treatment and supporting the physician in selecting the most effective course of action. In this study, we found that the relationship between the Rapid urease test and PCR appears with 94.6% sensitivity and 97.3% specificity, making it a highly sensitive test in comparison to PCR. This is comparable to another study that discovered that the rates of H. pylori detection by RUT, RDT, culture, and PCR were, respectively, 66.5%, 69.5%, 71%, and 67.5% with no discernible difference [20] Primary isolation of H. pylori from a biopsy specimen is a difficult process. The normal success rates for primary isolation of H. pylori from biopsy samples are reported to be in the range of 70% to 80% with 90% to 95% sensitivity and 100% specificity [21]. The organism is difficult to culture due to a number of uncontrollable circumstances, such as the organism's patchy distribution on the gastric mucosa, contamination of the biopsy forceps, the presence of oropharangial flora, the organism's viability being lost during transportation, etc. All of these could be to blame for the low negative predictive value of H. pylori culture [22,23]. For arapid urease test, stomach antral tissue samples for gastric biopsy were taken. Because there are fewer parietal cells and numerous H. pylori receptors in this area, the low acidity there enhances the possibility of detecting an organism's metabolic activity, such as the urease enzyme [24]. When the infected material turned red or pink, the test was deemed successful. After a short while, the majority of cases yielded favorable results. Patients with negative RUT results also had positive PCR results and a clinical presentation of H. pylori gastritis. Because two biopsies increase the sensitivity of the test, which necessitates at least 105 bacteria per milliliter, there may be a connection between the lack of positive RUT results for these individuals and the sample size [25].

Another hypothesis is that elderly people are more likely to have intestinal metaplasia and atrophic gastritis, both of which are linked to lower H. pylori density. These conditions also increase the chance of sample error. According to the findings of a recent study by one team, this clinically significant observation may be explained by the gastrointestinal mucosa being exposed to a variety of substances, including blood. In an in-vitro setting, the study discovered that a mixture of bile, gastric juice, and blood decreased the sensitivity of three separate RUTs.

Molecular study

The gyrA gene was used as a housekeeping gene in a conventional PCR to amplify DNA taken from the biopsy in order to detect H. pylori. The earlier method identifies particular genes, such as the very accurate gyr A gene for confirming H.

pylori infection [26]. The 108-bp region of the 23S rRNA gene in H. pylori is the target of the disclosed real-time PCR technique, which can identify two common SNPs A2134G and A2124G linked to clarithromycin resistance in the amplified product [27].

Distribution of clarithromycin resistant strain

The percentage of clarithromycin-resistant and sensitive strains, which appear to be 65.20% resistant and 34.80% sensitive, is considered a high resistance rate when compared to another antibiotic-resistant bacterium, such as MRSA, which was studied by Alsaimary [4] , who discovered 19.4 % from HCWs' noses.

In line with other studies that have demonstrated a strong and significant correlation between clarithromycin resistance and the failure of treatments based on the drug, with resistance reducing the effectiveness of treatments by more than 50%. The 23s RNA gene alterations that render the bacteria resistant to clarithromycin may be the source of this [28-30].

In Iran, resistance to clarithromycin is increasing in H. pylori strains, which in turn may lead to treatment failure [31]. A2142G, A2143G, or A2142C point mutations in the 23S rRNA of H. pylori, which reduce the affinity of the clarithromycin-resistant strain binding to the ribosome, may be connected to CAM resistance. The A2143G point mutation is the main contributor among the three point mutations [32,33] . In our study, A2142G appear as more than A2143G point mutation which may be due to the presence of other sub types with in the 23s RNA which leads to differences in the pathogenicity of the bacteria from region to region.

Epidemiology of clarithromycin resistance

This study explains that there is no relationship between clarithromycin resistance and the age of the patients, which resembles a study from Pakistan that found no association between clarithromycin resistance and age or sex [34] and agrees with a study from [35], who show that the proportion of heteroresistant infections in resistant cases was found to be independent of age and sex. However, our data disagree with the claims made by [36] that age can affect the eradication rate. We found that the eradication rate is higher in elderly patients with atrophic gastritis and reduced stomach acid output.

The doctor should do an antibiotic sensitivity test prior to initiating antibiotic treatment, according to an Alsaimary [4] study on lactamase-producing and non-producing Staphylococcus aureus. The majority of the tested drugs exhibited a rise in resistance with aging. These findings imply that while administering antibiotics, practitioners should take into account the patient's age and the locations of the infection.

Due to reduced blood rate supply to the stomach mucosa and promotion of acid secretion, smoking has been linked to the failure of H. pylori eradication. Additionally, smoking causes acid release, which decreases the effectiveness of acid-sensitive medications (e.g., amoxicillin) [24]. Another study indicated that smoking greatly increased the risk of first-line therapy for H. pylori infection failing [37]. In contrast to Eusebi et al., who found that cigarette smoking, alcohol consumption, diet, occupational exposure, and individual genetic trait have been demonstrated as risk factors associated with infection of H. pylori, due to the fact that the acid-stable antibiotic clarithromycin was not impacted by the increase in acid production, we didn't find a correlation between current cigarette smoking and eradication failure in the current investigation.

Distribution of clarithromycin resistant strain according to Diabetes status

The lack of a relationship between the clarithromycin-resistant strain and diabetes in this study may be due to the small number of cases with diabetes, so our data may not provide an accurate picture of the relationship between diabetes and the clarithromycin-resistant strain.

According to a 2009 study by Demir et al., type 2 diabetics had significantly lower H. pylori eradication rates and significantly higher levels of clarithromycin resistance. These findings may be related to factors like lowered immunocompetence, increased antibiotic resistance due to frequent antibiotic use, and subpar gastric absorption.

Sequencing

Sequencing success is influenced by a number of variables. These include both technical and analysis-related concerns. The purity of DNA isolation is crucial among the technical issues. According to the results of the PCR amplification, the genomics DNA isolation kits looked to be of good quality. In order to prevent DNA deterioration, it is crucial to preserve it at -20°C for an extended period of time [1]. The Neighbour-Joining approach was used to infer the evolutionary history [38-40]. The bootstrap consensus tree created from 500 replications is taken to represent the evolutionary history of the species under study [41-43]. The Jukes-Cantor technique was used to calculate the evolutionary distances [44-46] and are expressed in base substitutions per site. The investigation included 24 nucleotide sequences with Helicobacter pylori strain LIM-001 as an ancestral strain and the corresponding area of the gyrA gene. First, second, third, and noncoding codon locations were covered. All positions with gaps and missing data were eliminated. The total number of sites in the final dataset was 501. Evolutionary analyses were performed with MEGA7 [47-49].

CONCLUSION

The study concluded, that most ofH. pylori isolate from Basrah, Iraq, was determined to be resistant to clarithromycin due to a mutation in the 23sRNA gene, and there is no correlation between clarithromycin resistance and age, smoking, diabetes, or clinical diagnosis.

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