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# Determination of Antibiotic Resistance and Relevance of 23SRrna Gene in Helicobacter Pylori with Clarithromycin Resistance in Chronic Gastritis

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**Abstract**

Anti-toxin opposition of Helicobacter pylori (H. pylori) to treatment is going up. The clarithromycin obstruction is the serious issue of treatment disappointment in H. pylori disease .There is a basic need to decide the ebb and flow paces of such obstruction before authoritative treatment.We intended to decide antimicrobial opposition paces of H. pylori and impacts of point transformations during the 23S rRNA quality in H. pylori on clarithromycin opposition among the Egyptian populace, as a necessary advance prior to starting treatment.Fifty patients with H. pylori-positive ongoing gastritis were analyzed. H. pylori was distinguished by H.pylori stool antigen and microbiological refined of gastric biopsy . Absolute DNA was extricated from gastric biopsy examples and A2142G and A2143G transformations were recognized by PCR-limitation section length polymorphism (PCR-RFLP).H. pylori obstruction rates to clarithromycin , metronidazole, amoxicillin, and antibiotic medication were 54%, 28%,20% and 4% respectively.Additionally, for clarithromycin point changes in 23S rRNA types A2142G and A2143G of H. pylori were surveyed by PCR-RFLP measure, freak qualities were discovered to be 92.6 % of clarithromycin obstruction. A2143G change was recognized in 74.1% of tests and A2142G transformation in 18.5%. There was a solid relationship among MICs and point transformations in 23S rRNA gene.A2143G change was unmistakable . H. pylori conveying A2142G change indicated significantiy more elevated levels of MIC values for Clarithromycin anti-microbial. PCR-RFLP has uncovered a solid test permitting a fast identification of clarithromycin-obstruction. This is helpful in our nation where there is high pervasiveness of clarithromycin-obstruction prior to picking ideal treatment for H. pylori destruction.

**Keywords:** Clarithromycin resistance, A2142G, A2143G, 23S rRNA gene,Helicobacter pylori**.**

## 1.Introduction

Helicobacter pylori (H. pylori) is a Gram-negative bacterium that speaks to the essential driver of gastric malignant growth among patients with persistent gastritis if untreated [1].

The destruction treatment of H. pylori doesn't just mend gastritis of peptic ulcer sickness (PUD) yet it likewise forestalls the spread and repeat of disease and decreases the danger of improvement of gastric malignant growth; subsequently sparing further costs needed for therapy [2.]

In Egypt, the standard treatment for H. pylori diseases incorporates PPI and metronidazole with one anti-infection; that is either clarithromycin or amoxicillin [3]. The reasons for treatment disappointment in H. pylori diseases can be ordered into microorganism-related components, have related elements and treatment-related elements [4].

Clarithromycin is a macrolide anti-toxin that hinders protein blend of microorganisms by official to the 50s subunit of bacterial ribosomes. Corrosive dependability and great assimilation in the gastric mucosa renders it a decent decision for H. pylori annihilation when managed in high portions [5]. Some H. pylori strains have created protection from clarithromycin by change of the hereditary grouping in the peptidyltransferase circle of the 23S rRNA. H. pylori protection from clarithromycin is for the most part because of an adenine-to-guanine (A-G) progress at positions 2142 and 2143 and to an adenine-to-cytosine (A-C) transversion at position 2142 [6].

The fix pace of PUD is somewhere in the range of 0 and half when the H. pylori strain included is impervious to clarithromycin, while it is around 90% when the strain is powerless [6].

Clarithromycin-safe H. pylori depending on antimicrobial vulnerability tests as plate dissemination or E-test, requires further subculturing ventures for a few days [7] The requirement for quick opposition screening methodology that supplant the traditional social techniques is obligatory as H. pylori is a demanding moderate developing bacterium; hence culture-based vulnerability testing is a tedious and testing task [8].

Various sub-atomic based procedures have been prescribed as potential options in contrast to regular H. pylori location due to their high affectability, quick outcomes and exactness; despite the fact that at a more exorbitant cost [9].

## 2.Material and methods

### 2.1Study design

The present study was conducted on 50 Egyptian patients infected with H. pylori. They were all submitted to upper GIT endoscopy at the Gastroenterology Unit of Internal medicine Department ofBanha Faculty of medicine ,Banha university in the duration from June 2019 to June 2020.

### 2.2Inclusion and exclusion criteria

The study included patients Positive for H. pylori infection; documented by a previous H pylori stool

antigen test. No prior administration of antibiotics, bismuth, probiotics, H2 receptor antagonists, or PPIs in the immediately preceding 4 weeks.

patients with serious liver, kidney, heart, brain, lung or endocrine disorders,patients treated with gastrectomy , gastric angioplasty, or vagus nerve amputation and patients with malignant tumors or Zollinger-Ellison syndrome were excluded from the study.

An informed consent was obtained from all enrolled subjects and the study was approved by the ethics committee of BanhaUniversity. Full history was taken from all cases covering their complaint and clinical condition and previous history of treatment of gastric ulcer or H. pylori infection.

Upper GIT endoscopic examination was done to all subjects and gastric biopsy specimens were taken from the antrum and body of the stomach for PCR assay (one tissue biopsy) and for microbiological examination (one tissue biopsy).

**2.3Clinical isolates for culturing**

Gastric biopsies were preserved at 2 to 8°C in sterile saline for a maximum of 4 h until processing.Colony isolates were cultured on colombia agar with DENT supplementand then incubated in microaerophilic conditions at 37 c for 3-5 days in CO2 incubator. Microaerophilic conditions were maintained with the Anoxomat Mark III (MarkMicrobiology) [10].

- **Identification of H. pylori**

After incubation, small transparent colonies were selectedand tested by Gram stain (Gram-negative curved rods), oxidase(positive), catalase (positive), and rapid urease tests(positive) for confirmation [10].

- **Antibiotic Clarithromycin susceptibility testing**

To evaluate the susceptibility to clarithromycin, the minimuminhibitory concentration (MIC) were tested by E test.This was done on Muller Hinton agarenriched with 7% sheep blood [11].CLR resistance was defined according to the Clinical and Laboratory Standards Institute (CLSI) approved breakpoint (≥1.0 mg/L) [12].

- **Antibiotics (amoxicillin ,metronidazole and Tetracyclines ) susceptibility testing**

Disk diffusion method against different antimicrobial agents (amoxicillin ,metronidazole and Tetracyclines ) was done for all Helicobacter isolates. Results were recorded as resistance according to the following interpretive criteria: for Metronidazole a zone≥ 8 μg/mL and for Amoxicillin, a zone≥ 0.5 μg/mL. These break points were used based on the recommendations of the NCCLS.[13]While for Tetracycline it was ≥ 1 μg/mL. [14]

**2.4 Clinical isolates forH. pylori DNA extraction, amplification and PCR/RFLP**

**DNA extraction principle**

Gastric biopsy samples were digested with Proteinase K in the Lysis Solution. The lysate is then mixed with ethanol and loaded on the purification column where the DNA binds to the silica-gel membrane which binds DNA specifically in the presence of the guanidine-based lysis buffer, while PCR inhibitors such as polyvalent cations and proteins are removed during subsequent washes, leaving DNA in the eluent.

Purity was measured using the Nanodrop spectrophotometer. A ratio of ~2.0 is generally accepted as “pure” for DNA.

**2.5Molecular detection of H. pylori by PCR**

DNA amplification was done using my Taq red mix (2x) PCR Master Mix Kit supplied by Qiagen ( USA, catalogue number BIO-25044) on the extracted DNA . It was used in the PCR reactions with the oligonucleotides:Hp23Sr6 sense (5′CACACAGGTAGATGAGATGAGTA3′) and Hp23Sr7 antisense (CACACAGAACCACCGGATCACTA3′), which amplified a fragment of 768pb corresponding to the domain V of the H. pylori 23S rRNA.

PCR condition was 94°C 5′ followed by 40 cycles of 94°C 30″/60°C 30″/72°C 30″ and one cycle at 72°C 7′, with a total volume of 25 μl containing 1× PCR buffer, 200 μMdNTPs, 2.0 mM MgCl2, 1 μM of each oligonucleotide, 1.25 U Taq DNA Polimerase Platinum Brazil (Invitrogen).

**2.6Molecular detection of clarithromycin resistance**

By PCR-RFLP The amplified fragments were digested with MboII and BsaI (New England Biolabs). These enzymes distinguish mutations in the H. pylori domain V of the 23S rDNA at the positions 2142 and 2143, respectively. In the presence of A2142G mutation the resulting restriction DNA fragments are of 418 bp and 350 bp and in the presence of A2143G mutation the resulting fragments are of 108, 310 and 350 bp. The products of PCR reactions and restriction analysis were resolved in 1.5% agarose gels, stained with ethidium bromide and photographed under UV light[15].

**2.7Statistical methods**

This study was conducted on a chronic gastritis case sample of 50 patients. The data were categorized in respect to the patients, gender and age groups.Data were analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). Two types of statistical analysis were conducted. Student’s t-test and Mann-Whitney test were used to compare the quantitative variables. Chi-square ($\chi 2$) test and Fisher's Exact Test were used to compare the qualitative variables. All these tests were used as tests of significance at $P<0.05$.

**3. Results**

A total of 50 patients suffering from upper GIT symptoms were enrolled . All the participants were positive for H. pylori Ag in stool and were submitted to upper GIT endoscopy. 27/50 (54%) of the clinical H. pylori isolates were resistant to Clarithromycin (CLR) using E-test while the resistance to Metronidazole, Amoxicillin (AMX) and tetracycline was (28%, 20% and 4%) respectively by using disc diffusion Fig (1)

Among the H. pylori isolates, 6/50 (12%) were resistant to both metronidazole and Amoxicillin, 6/50 (12%) were resistant to both clarithromycin and Amoxicillin followed by 5/50 (10%) resistant to both clarithromycin and Metronidazole, only 2/50 (4%) were resistant to three antibiotics (Clarithromycin (CLR), Metronidazole and Amoxicillin (AMX).

Table (1)Clarithromycin susceptibility by E test showed that 27/50 patients (54%) were infected with H. pylori resistant strains and 23/50 patients (46%) were infected with H. pylori Susceptible strains. There was no statistically significant difference between the two studied H pylori isolates regarding to age (P value > 0.05). While, there was statistically significant difference (P value < 0.05) between the two studied isolates as regard sex , mutations in V domain of 23s rRNA gene and MIC.The frequency of mutations in 23s rRNAgene was 92.6% and 4.3% for Resistant and Susceptible isolates respectively. Among resistant isolates the frequency of mutations in 23s rRNAgene was 74.1% and 18.5% for A2143 G and A2142 G respectively.

There was no statistically significant difference between the two studied mutated isolates regarding to age (P value > 0.05). While, there was statistically significant difference between the two studied mutated isolates as regard sex (P value < 0.05), the frequency of A2143 G mutations was higher in females. A2143G mutation was more frequent in CLR-resistant isolates than A2142G mutation. Table (2)

H. pylori isolates carried A2142G mutations showed significant higher levels of MIC values for Clarithromycin antibiotic than isolates carried A2143G mutationsFig (2).

**Table (1)**Relation between clarithromycin (CLR) antibiotic and different parameters (n = 50) .

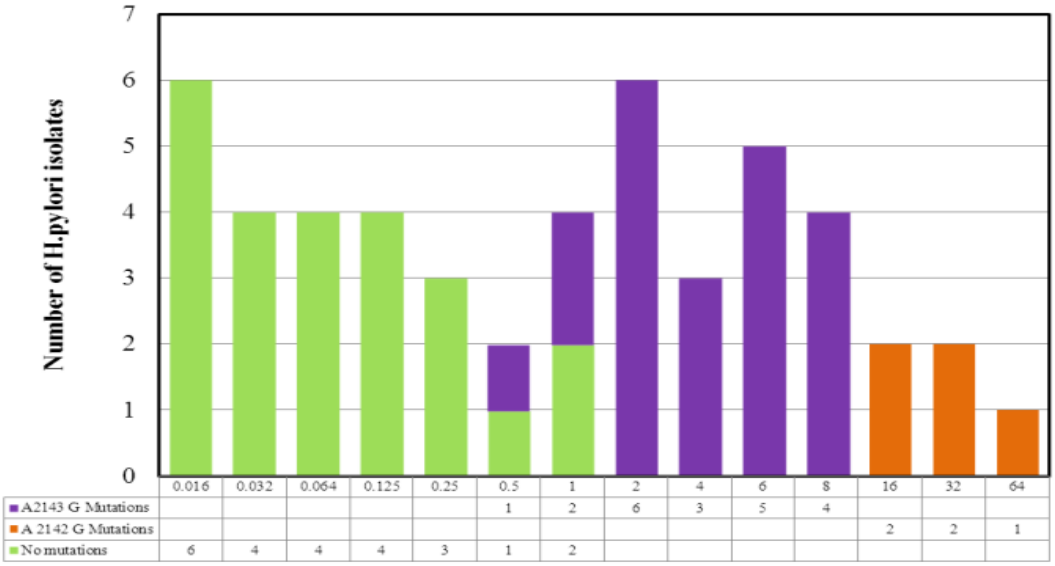
| | Clarithromycin (CLR) antibiotic | | | | Test of Sig. | p |
|---|---|---|---|---|---|---|
| | Resistant strains (n = 27) | | Susceptible strains (n = 23) | | | |
| | No. | % | No. | % | | |
| **Age** | | | | | | |
| **<30** | 11 | 40.7 | 9 | 39.1 | $\chi^2$= 0.016 | 0.992 |
| **30 – 40** | 9 | 33.3 | 8 | 34.8 | | |
| **≥40** | 7 | 25.9 | 6 | 26.1 | | |
| **Min. – Max.** | 20.0 – 45.0 | | 20.0 – 43.0 | | t= 0.268 | 0.790 |
| **Mean ± SD.** | 32.33 ± 8.19 | | 32.91 ± 6.87 | | | |
| **Median (IQR)** | 31.0 (25.50 – 39.50) | | 33.0 (27.0 – 39.50) | | | |
| **Sex** | | | | | | |
| **Male** | 10 | 37.0 | 16 | 69.6 | $\chi^2$= 5.265* | 0.022* |
| **Female** | 17 | 63.0 | 7 | 30.4 | | |
| **MIC (ug/ml)** | | | | | | |
| **Min. – Max.** | 1.0 – 64.0 | | 0.02 – 0.50 | | U= 0.0* | <0.001* |
| **Mean ± SD.** | 9.26 ± 13.66 | | 0.12 ± 0.14 | | | |
| **Median (IQR)** | 6.0 (2.0 – 8.0) | | 0.06 (0.02 – 0.13) | | | |
| **Gene mutation** | | | | | | |
| **No mutations in 23srRNA gene** | 2 | 7.4 | 22 | 95.7 | $\chi^2$= 38.749* | <0.001* |
| **Mutations in 23srRNA gene** | 25 | 92.6 | 1 | 4.3 | | |
| **No mutations in 23srRNA gene** | 2 | 7.4 | 22 | 95.7 | $\chi^2$= 42.487* | MCp <0.001* |
| **A2143 G mutations** | 20 | 74.1 | 1 | 4.3 | | |
| **A2142 G mutations** | 5 | 18.5 | 0 | 0.0 | | |

$\chi^2$: Chi square test MC: Monte Carlo t: Student t-test U: Mann Whitney test
p: p value for association between different categories *: Statistically significant at p ≤ 0.0

**Table(4)**Relation between the presence of A2143 G and A 2142 G in H. pylori 23s rRNA gene (n = 50).

| Antibiotic | Mutations in 23srRNA gene | | | | Test of Sig. | p |
|---|---|---|---|---|---|---|
| | A2143 G (n = 21) | | A 2142 G (n = 5) | | | |
| | No. | % | No. | % | | |
| **Age** | | | | | | |
| **<30** | 9 | 42.9 | 1 | 20.0 | $\chi^2$= 1.188 | $^{MC}$p= 0.700 |
| **30 – 40** | 7 | 33.3 | 2 | 40.0 | | |
| **≥40** | 5 | 23.8 | 2 | 40.0 | | |
| **Min. – Max.** | 20.0 – 45.0 | | 29.0 – 44.0 | | t= 1.206 | 0.240 |
| **Mean ± SD.** | 31.67 ± 8.55 | | 36.60 ± 6.35 | | | |
| **Median** | 33.0 | | 39.0 | | | |
| **Sex** | | | | | | |
| **Male** | 5 | 23.8 | 4 | 80.0 | $\chi^2$= 5.634* | $^{FE}$p= 0.034* |
| **Female** | 16 | 76.2 | 1 | 20.0 | | |
| **MIC (ug/ml)** | | | | | | |
| **Min. – Max.** | 0.50 – 8.0 | | 16.0 – 64.0 | | U= 0.0 | <0.001* |
| **Mean ± SD.** | 4.21 ± 2.62 | | 32.0 ± 19.60 | | | |
| **Median** | 4.0 | | 32.0 | | | |
| **Single resistant to CLR** | 20 | 95.2 | 5 | 100.0 | $\chi^2$=0.248 | $^{FE}$p=1.000 |

$\chi^2$: Chi square test FE: Fisher Exact MC: Monte Carlo t: Student t-testU: Mann Whitney test p: p value for association between different categories *: Statistically significant at p ≤ 0.05




**Fig(1)**Distribution of H.pylori isolates according MICs and 23S rRNA mutations.

## 4.Discussion

H. pylori the board in the clinical practice stays a test for the doctors [16].

The current treatment of H. pylori is observational. In this way, the objective in planning a treatment routine should zero in on a methodology which brings about a fix rate moving toward 100% [17].

Culture has practically 100% explicitness however even experienced labs recuperate the bacterium from simply half to 70% of tainted biopsies [18]. Then again, PCR-based sans culture procedures are exceptionally exact in identifying even negligible hints of genotypically safe strains [19].

Among the examined H. pylori gastric biopsy examples, we detailed impressive phenotypically safe arguments against Metronidazole, Amoxicillin (AMX) and antibiotic medication which were (28%, 20% and 4%) individually utilizing plate dissemination technique. Likewise,( Diab et al., [20] . reported extensive obstruction against metronidazole (25%) and amoxicillin (18.3%) utilizing traditional PCR test followed by sequencing of DNA parts of rdxA and Pbp1A qualities individually and little protection from antibiotic medication utilizing ongoing PCR for recognition of 16S rRNA.

Two past Egyptian investigations demonstrated wide variety in frequencies of metronidazole opposition rates; Zaki et al., [21] proposed lower rate (12.8%) of protection from metronidazole, while

Fathi et al., [22]. announced that solitary 25% of confines were negative for rdxA quality by PCR, although100% of cases were phenotypically impervious to metronidazole by plate dissemination and E-test strategies.

Commonness of essential clarithromycin obstruction is expanding around the world. Three central matter transformations in 23S rRNA at A2143G, A2142G, A2142C of area V are liable for over 90% of clarithromycin opposition cases, and the most well-known is A-G changes at position2143 (A2143G) [23] .

As to vulnerability by E test played out, the clinical H. pylori detaches were impervious to Clarithromycin (CLR) 27/50(54%). By utilizing PCR/RFLP strategy in our examination, we found that the pervasiveness of clarithromycin obstruction was 25/50(50%) because of the point transformations at area V of the H. pylori 23S rRNA. Comparative outcomes have been accounted for by Ghaith et al., [24].

The current outcome was not in accordance with a past reports in Egyptian investigations Alhammad et al., [25] , Zaki et al. [21], and Diab et al., [20] who recorded that obstruction rates to clarithromycin were (26%, 22.4% and 6.7%) individually by utilizing constant PCR for discovery of 23S rRNA.

The as of now recorded high pace of protection from clarithromycin could be identified with cross-reactivity with different macrolides as opposed to past utilization of clarithromycin, as it is a costly anti-infection and not regularly utilized in Egypt for treatment of respiratory plot contamination [25].

In the current work, the protection from CLR didn't measurably vary with expanded age (P esteem > 0.05) , while the obstruction rate was factually altogether higher in females,these outcomes concurred with De Francesco et al., [26] who announced female transcendence of clarithromycin safe H. pylori strains.

This didn't coordinate with Alhammad et al., [25] who found that 46.2% of clarithromycin safe strains of H. pylori were recognized in the gathering of patients ≥ 40 years old. Improbable, sex didn't have a huge relationship with the pervasiveness of protection from clarithromycin. Though, Diab et al., [27] announced male prevalence .

In our examination clarithromycin weakness (MIC) was dictated by E-test. To distinguish transformations related with protection from clarithromycin we enhanced two areas of 23S rRNA. There was critical connection between presence of changes in V area of 23s rRNA quality in H. pylori confines and CLR obstruction (P esteem < 0.05). While, there is no relationship between presence of such changes in H. pylori confines and obstruction for different anti-infection agents.

Our examination likewise affirmed a solid relationship among MICs and point changes in 23S rRNA quality. The recurrence of changes in 23s rRNA quality was 92.6 (25/27)% and 4.3 (1/23) % among Resistant and Susceptible disconnects respectively.The serious level of relationship between clarithromycin opposition in H. pylori and point transformations in the peptidyltransferase of 23S rRNA quality proposes that sub-atomic testing will be helpfully clinically and empower defenselessness testing without requirement for culture from biopsy tests.

Unfortunally, we discovered little error among phenotypic and genotypic strategies as one confine holding genotypic opposition that doesn't arise phenotypically; this distinction is fundamentally because of a heteroresistant status, [28]. Moreover; two duplicates of the 23S rRNA quality exist in H. pylori chromosomal DNA, and a change in one duplicate has been demonstrated to be sufficient to present clarithromycin opposition. In fact, the transformations might be heterozygous. Notwithstanding, in view of the evident uncommonness of heterozygosity, the blend disease or heteroresistant status is in all probability [29].

Our examination likewise affirmed two segregates with phenotypic obstruction and genotypic powerlessness as they didn't convey point transformations in 23S rRNA quality, these dissonant outcomes may happen because of the presence of point changes not explored or other hereditary components, for example, efflux siphons or RNA methylation (Liu et al., [30]; De Francesco et al., [28]; Hirata et al., [31] ).

In the current investigation transformation site of 23S rRNA related with clarithromycin opposition was resolved as A2143G in the larger part 20/27(74.1%) of H. pylori safe cases while A2142G in the minority 5/27(18.5%) of H. pylori cases.

These outcomes were predictable with information gotten from other Egyptian investigation as Alhammad et al., [25] who detailed that, transformation site of 23S rRNA related with clarithromycin opposition was resolved as A2143G in the lion's share (77%) of H. pylori strains . Notwithstanding that, Vala et al., [32].and Diab et al., [20].detected A2143G point transformation in 100% of H. pylori strains recuperated from their examined patients in Iran and Egypt, individually. Additionally, Acosta et al., [33].announced that A2143G transformation was more incessant than A2142G change.

Our outcomes were not in concurrence with the investigation of Ghaith et al., [24] who reported that, point transformation A2142G recognized by MboII protein, while A2143G change was not identified in any segregate as the intensified DNA item was not processed by BsaI. Also, Hansomburana et al., [34] reported that A2142G was more regular than A2143G changes. However, Klesiewicz et al., [35].discovered that the commonest transformations were A2143G and A2142G changes and they had a similar rate in all confines tried.

In this examination, Female patients had a higher occurrence of the 2143G clarithromycin safe genotype than male patients, despite the fact that the positive pace of H pylori disease was not distinctive between genders. This was in a similar line with Park et al., [36].

**5.Conclusion**

The high pervasiveness of opposition of H. pylori clinical disengages to clarithromycin (54%) because of the point changes at area V of the H. pylori 23S rRNA (particularly A2143G transformation) may command changing of the standard clarithromycin containing triple treatment. The report serves an update that a severe anti-microbial utilization strategy is required in our nation where H. pylori predominance was ascending.

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