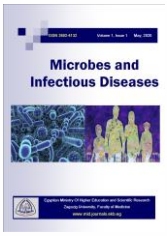
Microbes and Infectious Diseases 2025; 6(3): 3051-3064



Microbes and Infectious Diseases

Journal homepage: https://mid.journals.ekb.eg/

Original article

Molecular detection of clarithromycin-resistant *Helicobacter pylori* in stool samples using real-time PCR

Menna Allah Samir Mohamed*[1], Rania Mohamed Abdelhalim[1], Névine Nabil Kassem[1], Eman Mohammed Kamel[1], Sally Mohammed Saber[1], Engy Yousry Elsayed[2], Samar Saad Mohamed Rashad[1]

1- Clinical Pathology Department, Faculty of Medicine, Ain Shams University, Egypt.

2- Internal Medicine Department, Faculty of Medicine, Ain Shams University, Egypt.

ARTICLE INFO

Article history:
Received 28 December 2022
Received in revised form 18 January 2023
Accepted 24 January 2023

Keywords:
Molecular identification
Helicobacter pylori
Resistance to Clarithromycin

ABSTRACT

Background: *Helicobacter pylori* is a spiral-shaped bacterium that thrives in the digestive system. This bacterial infection is highly prevalent, it affects more than 50% of the world's population. It is common among children in developing countries. Transmission can occur through close personal contact. Factors that increase the likelihood of infection include residing in densely populated areas with limited access to clean water sources. Gene mutations in *Helicobacter pylori* can lead to clarithromycin resistance by altering the bacterial ribosomal target site. These mutations, particularly in the 23S rRNA gene, prevent clarithromycin from binding, allowing the bacteria to survive in the stomach. **Aim of Work:** This research seeks to identify point mutations linked to *Helicobacter pylo*ri's resistance to clarithromycin in stool samples through real-time PCR analysis, aiming to assist physicians in effectively treating patients infected with *H. pylori***. Methodology:** From December 2019 to May 2020, this cross-sectional investigation involved 90 patients exhibiting symptoms indicative of *Helicobacter pylori* infection. These individuals were examined at the endoscopy unit of Ain Shams University Hospitals' Hepatology, Gastroenterology, and Infectious Disease Department.
Results: prevalence rate of clarithromycin resistance (46%), with A2143G accounting for 28% and A2142G for 18%. **Conclusion:** The study found a high rate of clarithromycin-resistant *H. pylori* (46%), mainly linked to the A2143G point mutation, and also identified the A2142G point mutation. Neither age nor gender showed a significant association with clarithromycin resistance. Given these findings, clarithromycin-based *H. pylori* treatment regimen may not be advisable as an empirical treatment approach in Egypt**.** .

Introduction

Helicobacter pylori is a spiral-shaped microorganism that inhabits the digestive system. This bacterium is highly prevalent, potentially affecting over half of the global population. In less developed nations, *H. pylori* infection is particularly common among children. Transmission of *H. pylori* may occur through direct person-to-person contact. Living in crowded environments without access to clean water supplies is a significant risk factor for *H. pylori* infection [1].

In the Americas, the overall *H. pylori* infection prevalence stands at 50%. Despite a global downward trend, Africa maintains a notably high prevalence of 70% [2], the prevalence of infection in Egypt is 70% [3].

DOI: 10.21608/MID.2025.337736.2447

* *Corresponding author:* Menna allah Samir Mohamed
E-mail address: *mennaallahsamir@yahoo.com*

Although *H. pylori* infections are typically asymptomatic, they can cause peptic ulcers in about 10% of infected individuals. Gastritis caused by *H. pylori* leads to a combined acute and chronic inflammatory response. Resistance to clarithromycin (CLA) poses significant challenges in managing *H. pylori*, a known risk factor for conditions ranging from gastritis to gastric carcinoma, often aggravated by Epstein-Barr virus infection [4].

Although traditionally viewed as a non-invasive pathogen, *H. pylori* has been identified as a facultative intracellular bacterium that infects innate immune cells. It can disrupt phagosome maturation, which may help explain the challenges in eliminating the infection [1].

To evade the stomach's acidic environment, H. pylori employ its flagella to burrow into the stomach's mucus lining, reaching the underlying epithelial cells where acidity is lower. Additionally, *H. pylori* neutralize its surroundings by producing large quantities of urease, an enzyme that breaks down urea in the stomach into carbon dioxide and ammonia. These substances interact with the potent acids in the surroundings to create a neutralized zone in their vicinity [5].

H. pylori infection is diagnosed through endoscopy, molecular methods, and culturing. Non-invasive techniques include the urea breath test, antibody detection, and stool antigen test [6]. Molecular methods Sensitivity 95%, and specificity 100% much higher than that of histology and culture. the sensitivities of histology and culture were only 43% and 37% respectively [7].

All patients with a positive *H. pylori* test should be offered eradication treatment. This typically prevents ulcer recurrence and complications, even after discontinuing appropriate medications like proton pump inhibitors (PPIs). It also plays a crucial role in treating gastric lymphoma, although its use in preventing stomach cancer remains debatable [8].

A common first-line treatment is Clarithromycin (CLA)-based triple therapy, which combines Clarithromycin, a proton pump inhibitor (PPI), and either amoxicillin or metronidazole. However, *H. pylori*'s resistance to clarithromycin has been increasing steadily [9]. In European countries, the rate of primary *H. pylori* resistance to clarithromycin is 18%. while in the Eastern Mediterranean region, it's 33%. Africa shows an overall clarithromycin resistance of 15% [10].

Genetic analysis of resistant strains from patients who failed primary clarithromycin-based treatment revealed that mutations in the 23S rRNA are the main cause of resistance. Specifically, point mutations at positions 2143 (A to G) and 2142 (A to G or C) account for over 90% of clarithromycin resistance cases. The A2143G mutation is strongly linked to eradication therapy failure. The A2142G/C mutation has been reported less frequently [11]. Real-time PCRs have been employed to identify mutations associated with clarithromycin resistance in stool samples [6].

AIM OF THE WORK

This study aimed to detect point mutations associated with *Helicobacter pylori* clarithromycin resistance in stool samples through real-time PCR.with the goal of assisting clinicians in the appropriate management of *H. pylori* infected patients.

PATIENTS AND METHODS

Patients

This cross-sectional study involved ninety patients showing symptoms of *Helicobacter pylori* infection who visited the endoscopy unit at the Hepatology, Gastroenterology, and Infectious Disease Department of Ain Shams University Hospitals between December 2019 and May 2020.

Inclusion criteria:

1. Patients had clinical findings consistent with *Helicobacter pylori* infection: dyspepsia, dysphagia, weight loss, persistent epigastric pain, persistent vomiting, family history of gastric cancer, and iron deficiency anemia [12].

2. Recently diagnosed patients confirmed by conventional endoscopy and histological examination of gastric tissue obtained by endoscopy.

Exclusion criteria:

1. Patients treated with eradication therapy of *H. pylori* [13].

2. Patients with history of taking proton pump inhibitor (PPI) in the last two weeks [14].

3.Patients with history of taking antibiotics in the last four weeks [14].

Ethical consent:

Ethical alignment: "Written informed consent was obtained from all participants. and the study was approved by the medical ethics committee of Ain Shams University FWA 000017585.

Methods

Sample collection:

Stool samples were obtained in a clean sealable container.

Sample storage:

Each sample was divided into two parts. The first part was subjected to Rapid immunoassay on fresh stool samples. The second part of the sample was frozen at – 80º c for 2 months in clean container until Real-time PCR was done [15].

Stool samples were subjected to the following:

A. Rapid immunoassay for the detection of *H.pylori* antigens RAPID STRIP HpSA (Meridian, Italy) was done on stool samples of patients confirmed by histopathological examination [16].

B. Real-time PCRs to detect *H. pylori* mutations associated with clarithromycin resistance was done on stool samples with positive H.pylori stool antigen test [17].

C. Detection of *H. pylori* antigens by Rapid immunoassay RAPID STRIP HpSA (Meridian, Italy).

Principle of the Method:

RAPID STRIP HpSA Meridian is an in vitro qualitative procedure, based on a lateral flow chromatography technique that detects *H.pylori* antigens in human stool. It utilizes a monoclonal anti-*H. pylori* antibody. The strip is introduced in a tube containing diluted patient samples and the appearance of a pink- red line in the reading area indicated a positive result after 5 minutes of incubation at room temperature.

Interpretation of results:

a. Negative test results:

Only one blue coloured band (control line) appeared across the white central area of the reaction strip. *H.pylori* antigens are absent or below level of detection.

b.Positive test results:

In addition to the blue colored band (control line), another pink-red band (test line) also appeared across the white central area of the reaction strips (**Figure1**).

Detection of point mutations associated with clarithromycin resistance was performed by real time PCR through several steps which included:

1. Extraction of DNA from stool samples.
2. DNA amplification and High-Resolution Melt (HRM) analysis for the detection of 23SrRNA gene mutations (Rotor geneQ) (Qiagen, Germany)

1.Extraction of DNA from stool samples.

DNA was extracted from stool using QIAamp DNA stool mini kit supplied by QIAGEN, Germany.

a. Principle:

The QIAamp DNA stool mini kit was applied for rapid and efficient purification of DNA from stool samples. The DNA in the sample is liberated using proteinase K solution and lysis solution. Released DNA was bound exclusively and specifically to the spin filter surface. Denatured protein and other contaminants are removed with several washing procedures. The DNA is then eluted from the membrane with elution buffer.

Stool samples typically contain many compounds that can degrade DNA and inhibit downstream enzymatic reactions. To ensure removal of these substances, A special protocol is provided for isolating DNA from larger amounts of stool. The fast and easy procedure comprises the following steps:

- Lysis of and separation of impurities from stool samples in InhibitEX Buffer
- Purification of DNA on QIAamp Mini spin columns.

DNA amplification and High-Resolution Melt (HRM) analysis for the detection of 23SrRNA gene mutations (Rotor geneQ) (Qiagen, Germany)

The extracted DNA was amplified using 2x HRM PCR Master Mix and Analysis of Gene Mutations and Genetic Differences by HRM.

a. Principle:

Before performing HRM analysis, the target sequence was amplified to a high-copy number in the presence of the dsDNA-binding fluorescent dye, EvaGreen. The dye does not interact with ssDNA but actively binds to dsDNA and fluoresces brightly when bound. Change in fluorescence was used to measure the increase in DNA concentration during PCR and then to directly measure thermally induced DNA melting by HRM.

To perform high-resolution melting analysis, the temperature was increased from a lower to a higher temperature. The fluorescence of EvaGreen was measured continuously as the temperature was increased and is plotted against the temperature. EvaGreen fluoresces as long as it is bound to dsDNA. Due to the amplification procedure before the HRM analysis, fluorescence will be high at the beginning of the HRM analysis. Upon melting of dsDNA, EvaGreen is released, and the fluorescence is reduced to a background level. **Figure 2**.

Using the following primers [18]:

Forward: (5' – CAGTGAAATTGTAGTGGAGGTG – 3')

Reverse: (5' – CGCATGATATTCCCATTAGCAGT – 3')

b.Procedure

Real-time PCR was conducted using Rotor-Gene Q (Qiagen, Germany) under these conditions: initial denaturation at 95°C for 5 minutes, followed by 45 cycles of denaturation at 95°C for 10 seconds, annealing at 55 °C for 30 seconds and extension at 72°C for 10 seconds.HRM at 65-95°C.

c.Result interpretation

The PCR data file is imported into the HRM analysis software and the data was analyzed.

The presence or absence of the 23SrRNA gene point mutations was established. In such situations, the melting temperature difference between samples is due to sequence variation from the wild type. Wild type samples give the same shaped melt curve. However, if there is a mutation in the 23SrRNA gene, this will alter the temperature at which the DNA strands melt apart. So now the two melt curves appear different. The HRM machine has the ability to monitor this process in "high resolution", therefore identify if a mutation is present or not, **Figure 3.**

Statistical Analysis

Data was collected, revised, coded and entered to the Statistical Package for Social Science (IBM SPSS) version 23. The quantitative data were presented as mean, standard deviations and ranges when parametric. T-test and ANOVA test were used in comparison between quantitative variables.

Also, qualitative variables were presented as number and percentages.

The comparison between groups with qualitative data were done by using ***Chi-square test***.

The confidence interval was set to 95% and the margin of error accepted was set to 5%. So, the p-value was considered significant as the following:

- P > 0.05: Non-significant.
- P < 0.05: Significant.
- P < 0.01: Highly significant.

RESULTS

The study initially screened 90 patients, with 66 testing positive for *H. pylori* histologically; 50 stool samples were analyzed for clarithromycin resistance **(Table 1).**

Results of rapid strip *Helicobacter pylori* stool antigen test:

In our study, 50 out of 66 (76%) stool specimens of patients confirmed by histopthalogical examination had positive *H.pylori* stool antigen by rapid strip test **(Table 2).**

In our study out of 50 patients with positive stool antigen test, 27/50 (54%) were males and 23/50 (46%) were females. Their age ranged from 18 to 72 years, with mean ± standard deviation 40.2 ±12.6years. As in **(Table 3)**

Results of High-Resolution Melt analysis:

In our study, 27/50 (54 %) of stool samples revealed wild type, and 23/50 (46 %) were CLR-resistant isolates. Of the 23 clarithromycin-resistant isolates, A2143G accounted for 60%, and A2142G for 40%, reflecting global trends in mutation prevalence. This is demonstrated in Table (4), figure (4).

In our study clarithromycin resistant isolates were 23. Isolates showing A2143G point mutation were 14/23 (60%), while isolates showing A2142G point mutation were 9/23 (40%).

Relation of clarithromycin resistance to gender

There is no notable difference in the detection of clarithromycin resistance between males and females. In males, clarithromycin resistant *H.pylori* positive samples were 13/23 (56%), in females clarithromycin resistant *H.Pylori* positive samples were 10/23 (44%) as demonstrated in **Table 5.**

Relation of clarithromycin resistance to age

There is no statistically significant correlation between clarithromycin resistance and age. For age ≤29 years 1/23(4%) showed

clarithromycin resistance, for age 30 -60 years 18/23(79%) showed clarithromycin resistance. for age ≥60 clarithromycin resistance was 4/23(17%) as demonstrated in **Table 5**.

For age less than or equal 29 years, four patients were infected with wild strains of H.pylori, only one patient was infected with an A2142G mutation. For Age between 30 and 59 years, twenty patients were infected with wild strains, six were infected with *A2142G* mutation, and twelve patients were infected with A2143G mutation. individuals in this age group may have had more exposure to antibiotics over their lifetime. This age group might also have more chronic health conditions (e.g., respiratory issues, peptic ulcers) that require antibiotic treatment. For age more than or equal 60 years three patients were infected with wild strains, two patients were infected with A2142G mutation, and two patients were infected with A2143G.This is shown in **Table 6** and **Figure 5.**

Male patients infected with wild type of *H.pylori* were fourteen patient, those who were infected with strains having A2142G mutation were five patients, and those who were infected with strain having A2143G mutation were eight patients. Female patients infected with wild type of *H.pylori* were thirteen patient, those who were infected with strains having A2142G mutation were four patients, and those who were infected with strain having A2143G mutation were six patients this is demonstrated in **Table 7** and **Figure 6.**

Table 1. Endoscopy and Histopathological Diagnosis of *H. pylori.*

	Endoscopy N=90
Positive by histopathological examination	66 (73%)
Negative by histopathological examination	24 (27%)

Table 2. Results of Rapid Stool Antigen Test.

	Positive by histopathological examination N= 66
Positive stool antigen test	50 (76%)
Negative stool antigen test	16 (24%)

Table 3. Demographics of Patients with Positive Stool Antigen Test.

	Demographic data		**P value**	**Sig**
Age in years	**Mean ± SD**			
	40.2 ±12.6			
Gender (%)	Male	Female	0.3	NS
	27 (54%)	23 (46%)		

Table 4. Percentage of wild type and clarithromycin mutation detected.

	N= 50	**%**
Wild type	27	54%
A2143G mutation	14	28%
A2142G mutation	9	18%

Table 5. Association between age, gender and clarithromycin resistance in *H.pylori***.**

		Wild N= 27 (%)	**Clarithromycin resistance N= 23 (%)**	**P value**	**Sig**
Age	≤ 29	4 (15%)	1 (4%)	0.419	NS
	30-59	20 (74%)	18(79%)		
	≥ 60	3 (11%)	4 (17%)		
Gender	male	14 (52%)	13 (56%)	0.741	NS
	female	13(48%)	10 (44%)		

Table 6. Association between different age groups and clarithromycin resistant genes.

	Wild N= 27	**A2142G N= 9**	**A2143G N= 14**	**Total clarithromycin resistant N=23**	**P Value**	**Sig**
≤29	4 (15%)	1 (11%)	0	1	0.570	Ns
30-59	20 (74%)	6 (67%)	12 (86%)	18		
≥60	3 (11%)	2 (22%)	2 (14%)	4		

Table 7. Association between gender and clarithromycin resistant genes.

Gender	**Wild N= 27**	**A2142G N= 9**	**A2143G N= 14**	**Total clarithromycin resistant N=23**	**P Value**	**Sig**
Male	14(52%)	5(56%)	8(57%)	13	0.944	NS
Female	13(48%)	4(44%)	6(43%)	10		

Figure 1. Rapid strip HpSA showing positive test results.

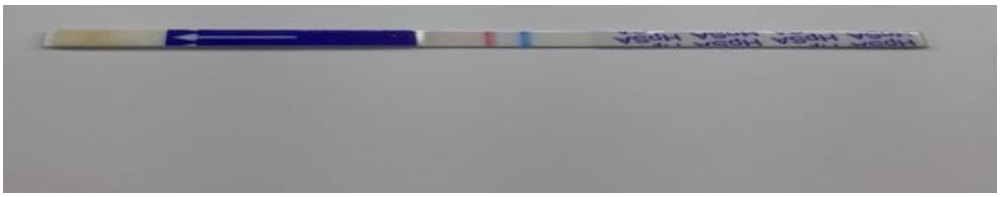


Figure 2. High resolution melt curve showing the high initial fluorescence when all products are double-stranded and the maximum amount of dye is bound. As the temperature increases, the PCR products denature, dye is released, and the fluorescent signal drops, the decrease in fluorescence starts slowly, but when the double-stranded DNA melts into its single-stranded, fully denaturated form, a sharp decrease in fluorescence is detected.

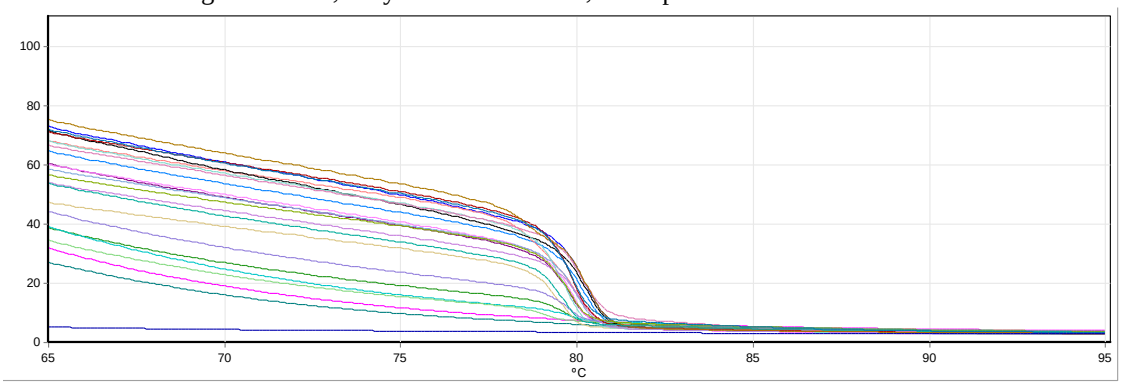


Figure 3. High Resolution melt curve with a single point mutation. each designated sequence is presented left to each curve.

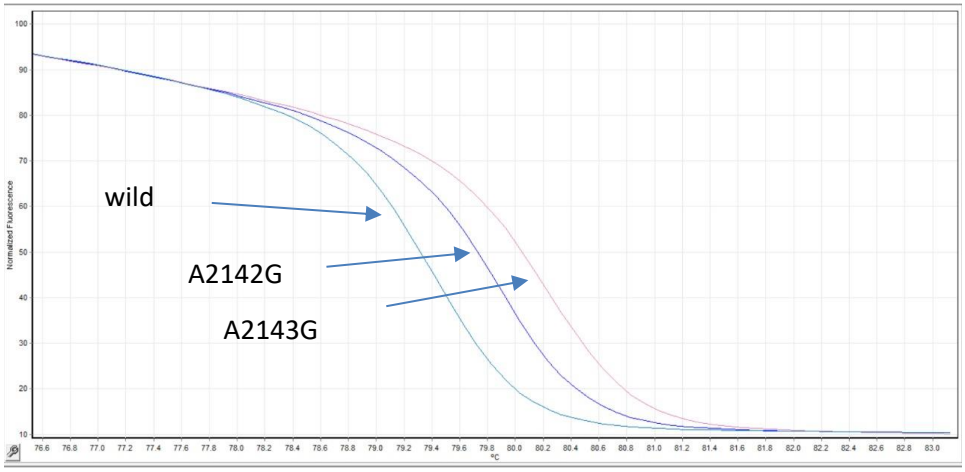


Figure 4. Percentage of wild type and clarithromycin mutation**.**

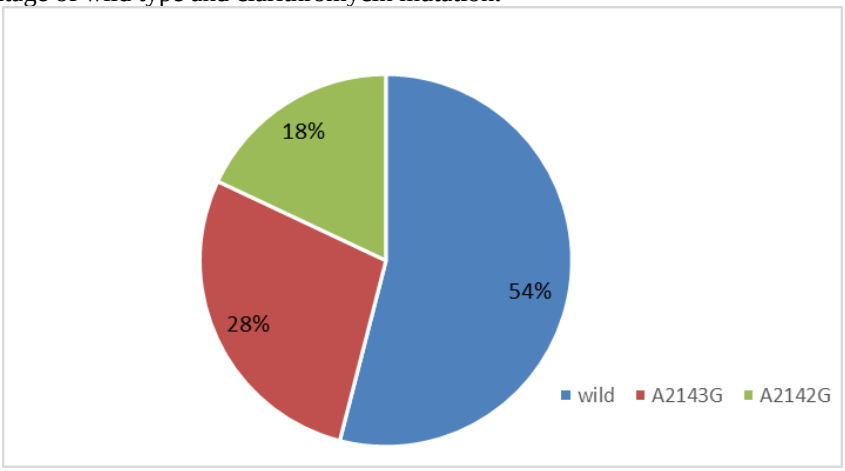


Figure 5. Association between different age groups and clarithromycin resistant genes.

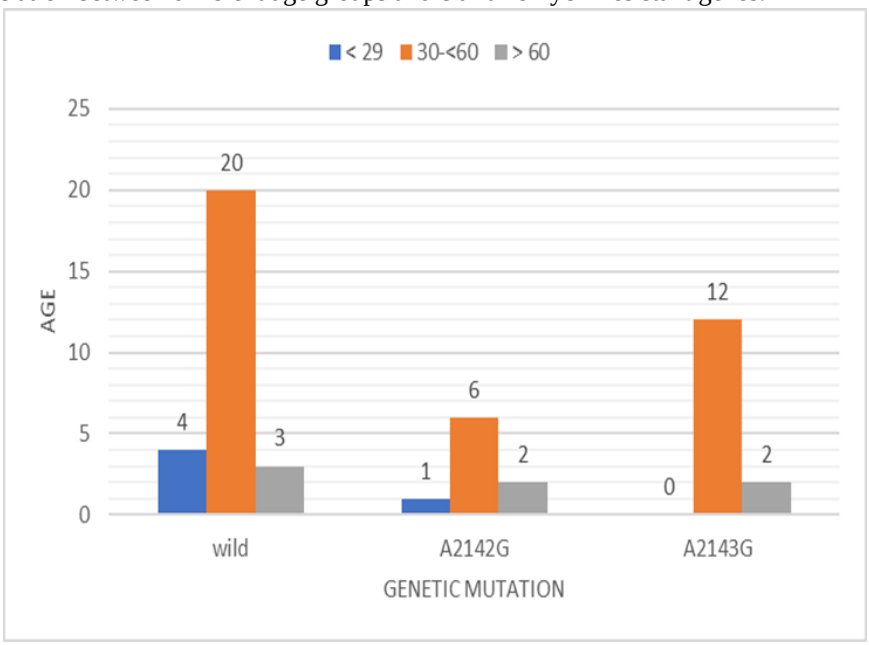


Figure 6: Association between gender and clarithromycin resistant genes.

Discussion

A research project at Babol University examined 61 individuals experiencing dyspeptic symptoms. *H. pylori* was identified in 38 (62.3%) gastric biopsy samples, with 25 (66%) of these also showing positive HpSA results, which aligns closely with our findings. The *H. pylori* stool antigen test offers numerous benefits compared to alternative methods. It serves both diagnostic and post-treatment monitoring purposes. This non-invasive, straightforward, quick, and cost-effective approach eliminates the need for laboratory visits (requiring only a stool sample) or fasting, which are necessary for endoscopy and urea breath tests [19].

In our present investigation, among patients with positive stool antigen tests, the average age (±standard deviation) was 40.2±12.6 years. The gender distribution showed 27/50 (54%) males and 23/50 (46%) females **(Table 3)**. According to [20], 53% were male patients [mean ± standard deviation (S.D.) age, 45 ± 17.9 years] and 47% were female patients (mean ± S.D. age, 41.5 ± 15.2 years). Another study [21] reported *H. pylori* positive patients were predominantly male (53.5%) compared to female (46.5%), mirroring our results. In the study done by Zamani [22], who noted a global *H. pylori* infection rate of 42.7% in females versus 46.3% in males.

But in the study done by Hulten [23] (38%) were male and the mean age of all patients was 46.4 ± 13 years which is different from this study**.**

Sex-differences in the prevalence of *H. pylori* infection may be explained by physiological differences, namely sex hormones (estrogens), influence the immune system. This results in a gender dimorphism in the immune function with females having higher immunoglobulin levels. Sex hormones may also affect immunity and the inflammatory response to *H. pylori* differently in men and women; these hormones can interfere, directly or indirectly, with the cell receptors altering immunological response [24].

In this study as regards clarithromycin (CLR) resistance, 46% of the isolates were CLR-resistant. The reported clarithromycin resistance is high due to high macrolide exposure, prescribing attitudes of physicians. In the study done by Bińkowska [25], reported the overall resistance rate to clarithromycin (46%). And [26] in Mansoura University Children Hospital, Egypt from December 2014 till August 2015, performed molecular determination of the Clarithromycin Resistance with PCR-RFLP Restriction fragment length polymorphism, reported resistance rate (46.2%) to clarithromycin**.** In the study done by Leonardi [27]**,** a total of 151 patients underwent 13C urea breath test, Bacterial DNA extraction from stool samples, Real time PCR and high-resolution melt analysis for the detection of 23S rRNA gene mutations: (49.1%) had a Point mutation associated with clarithromycin resistance. Metwally *et al* [28] performed a cross-sectional study included 134 adult patients with dyspepsia who attended the endoscopy unit of the Hepatology, Gastroenterology, and Infectious Diseases Department at Benha University Hospital between October 2018 and October 2020 Antibiotic susceptibility testing was done using disk diffusion. *H. pylori* clarithromycin resistance rates were (40%)**.**

In the study done by Hussein [29] 115 samples were collected from patients strongly suspected of *H. pylori* infection presented for upper gastrointestinal endoscopy in Iraq. Specimens were cultured on brain heart infusion agar containing various antibiotics and were incubated at 37 °C under microaerophilic conditions. For identification *of H. pylori.* Isolates biochemical tests and RT-PCR assay were applied. The Epsilometer test was used in the antibiotic susceptibility testing as dependent on the CLSI standard. The Restriction Fragment Length Polymorphism technique was used to determine point mutations. 69.1% of the specimens showed Clarithromycin resistance**.**

A hospital-based cross-sectional analytic study was conducted between October 2019 and October 2020 at the Gastroenterology Unit, Military Hospital, Yangon, Myanmar (South-East Asian country). A total of 98 *H pylori*-infected patients were involved and among them, genotypic clarithromycin-sensitive strain was 93.9% and clarithromycin-resistant strain was 6.1% mainly associated with A2143G point mutation which is different from this study**.** The rates of antibiotic resistance for *H.pylori* vary geographically [30]**.** The low clarithromycin resistant rate in H.pylori infection may also be due to low macrolide exposure[31]**.**

In Gehlot et al [20] study, Patients who underwent endoscopy at Yashoda Super specialty

Hospital (Ghaziabad, India) were enrolled in the study. Minimum inhibitory concentrations (MICs) were determined by the agar dilution method, and point mutations in clarithromycin-resistant strains were identified by PCR-restriction fragment length polymorphism (PCR-RFLP) and DNA sequencing. While Gehlot et al.reported 11.8%resistance rate, contrasting findings by Hulten et al. [23] showed resistance rates of 17.4%.

In Zagazig University Hospitals a study included 123 patients during upper gastro-duodenoscopy in the Endoscopy Units of. Clarithromycin resistance was assessed by Polymerase chain reaction-amplification and restriction fragment length polymorphism (PCR-RFLP) Clarithromycin resistance was detected in (66.19 %) positive *H. pylori* strains which is higher than our study. This indicates high prevalence of clarithromycin resistant *H. pylori* among Egyptian patients [21]**.**

Prevalence of clarithromycin resistance is high in developing world including Africa. This could impair the first line triple therapy of the *H.pylori* infection. There is a need of conducting surveillance of *H.pylori* susceptibility pattern in Africa for dual and triple resistance which can be used for the empirical treatment [32]**.**

The variation in the resistance to clarithromycin can be attributed to different sample size, age groups, population studied, and geographic variations which may greatly differ in antibiotic prescribing attitudes of physicians, and underlying resistance mechanisms [33].

In Europe, macrolide resistance rates ranged from 4.8% to 36.9% in 2018. A recent clinical trial in the US revealed a 23.2% clarithromycin resistance rate in the Eastern US, as determined by antimicrobial susceptibility testing (AST). Prior antibiotic use affects susceptibility, and the study advised considering antibiotic history when making treatment decisions. It also emphasized the importance of avoiding unnecessary antibiotics, aligning with the World Health Organization's strong recommendations, to enhance the chances of successful eradication. Both the Maastricht V/Florence and American College of Gastroenterology guidelines suggest that treatment regimens should be selected based on local resistance patterns.

In this study the point mutations A2143G in the 23S rRNA gene is the most predominant point mutation it was 60%, While A2142G was 40%. In the study done by Zaki[26] the most common type of mutation was for A2143G (53.4%) followed by A2142G (35.7%) which is nearly similar to this study.

In a study done by Abadi et al [35] a total of 147 consecutive patients infected with *H. pylori* were included. The (RFLP-PCR) method was applied to determine the frequency of point mutations in 23s rRNA gene. The Prevalence of A2143G was 93.7 % of the clarithromycin resistant strains, which is in consistent with this study**.** Also, this agrees with several studies who reported the most frequently occurring mutation in *H. pylori* strains resistant to clarithromycin was the A2143G mutation in the *23S rRNA* gene [20,36, 37].

On the other hand, in Zagazig a study reported The A2142G as the most frequent detected point mutations involved in clarithromycin resistance. Clarithromycin resistance was assessed by Polymerase chain reaction-amplification and restriction fragment length polymorphism (PCR-RFLP) [21].

In this study the agreement between sex and clarithromycin resistance was not significant, P value > 0.05 (table 5, 7). According to *Eghbali et al* [39] a total of 89 *H. pylori* strains were isolated from gastric biopsies of patients with gastric disorders such as gastritis, peptic ulcer, duodenal *ulcer*, non-ulcer dyspepsia and gastric adenocarcinoma. Isolated strains were tested for clarithromycin resistance. The Fisher exact test revealed that there was no significant relationship between clarithromycin resistance and gender (P >0.05), which is in consistent with this study**.** Also, several studies showed no observed association between gender and resistance of *H. pylori* isolates (P>0.05) [30, 35, 40].

In contrast to our study, Soltan and associates [21] reported significant association between clarithromycin resistance and gender with increase clarithromycin resistance among females (P< 0.05). Pathologically, females with H.pylori infection showed a lower degree of inflammation and lower activity scores in the antrum than males, which were associated with lower interleukin-8 production in the gastric mucosal samples of females than of males. Both microbial and host factors are critical for the clinical manifestation of *H.pylori* infection and eradication failure. The gender-based difference in the inflammatory response may influence the

eradication rate and accumulation of mutated *H. pylori* [41]**.**

In this study there was no statistically significant correlation between clarithromycin resistance and age, P value>0.05.

Abdollahi and associates [40]; Abadi and associates [35]; Soltan and colleagues [21]; Nyi and associates [30] found no associations observed between age and resistance status of *H. pylori* isolates (*P*>0.05).

According to ji and colleagues [42] A total of 29,034 gastric mucosa biopsy samples were randomly collected from January 1, 2009 to December 9, 2014 in Jiaxing City, China. An antibiotic susceptibility testing was determined using an agar-dilution method. Samples were subdivided into 7 groups (<20, 21–30, 31–40, 41–50, 51–60, 61–70, and 71–80 years of age). The higher clarithromycin-resistant group 31 to 50 year or 71 to 80 years of age, was found to have significant differences from the other resistance group which is different from our study.

In this study the age group between 30 to 59 years had the highest rate of clarithromycin resistance (79%). A retrospective observational study of clarithromycin resistance (Cla-res) involving 816 resistant cases out of 4744 *H. pylori*-infected patients from Central Hungary. The highest Clarithromycin resistant group was 30 to 59 years (55%) which is nearly similar to this study [31]. Such high resistance of strains to clarithromycin results mainly from excessive consumption of antibiotics, especially from the group of macrolides, and their widespread use in respiratory tract infections [38].

The high resistance significantly impacts treatment efficacy and necessitates adjustments in both clinical management and public health strategies. It led to reduced efficacy of Standard Therapies. Public health Strategies include monitoring antibiotic resistance patterns**,** which is essential to inform treatment guidelines and ensure the selection of effective empirical therapy [43].

Conclusion

Overall, this study highlights key findings regarding clarithromycin resistance and its clinical implications. It revealed increase rate of clarithromycin-resistant *H. pylori* (46%) and was associated with the A2143G point mutation and A2142G point mutation. The results of this study demonstrate the requirement for antibiotic susceptibility testing and molecular methods to detect point mutations before selecting drug regimens. This help to avoid treatment failure and ensure eradication of *H.pylori* after use of proper treatment. The advantages of Rapid Strip *H.pylori* stool antigen test. It is easy to perform, accurate, noninvasive, simple, rapid, used for diagnosis as well as monitoring after treatment, and inexpensive.

Recommendations

- PCR test is simple, convenient, rapid, non-invasive recommended test for detection of clarithromycin resistant genes.
- Treatment of H.pylori with clarithromycin should not be started except after testing for clarithromycin resistant genes.
- Testing for clarithromycin resistance could be challenging Strategies to address this could include: Point-of-Care Testing Development: Investing in portable molecular diagnostic tools tailored for low-resource environments, which are faster, cheaper, and easier to use. Partnerships and Collaborations: Partnering with international health organizations to subsidize costs, provide equipment, and establish supply chains for reagents and consumables. Batch Testing or Referral Labs: Establishing centralized laboratories that serve multiple clinics, reducing the need for each site to maintain advanced diagnostic capabilities. Selective Testing: Prioritizing resistance testing in patients with risk factors for resistance (e.g., prior macrolide exposure or areas with known high resistance rates).
- Current recommendations in Egypt suggest Alternative regimens, such as bismuth-based quadruple therapy, are preferred due to their higher efficacy in the context of prevalent antibiotic resistance
- It's essential for healthcare providers to stay informed about local antibiotic resistance patterns and to consider these trends when selecting treatment regimens for *H. pylori* infection.

- In future, vaccines may give a chance to prevent infection, and decrease gastric H.pylori colonization.
- Development of multivalent vaccines targeting several antigens to address strain diversity. Studies focusing on host-pathogen interactions to better understand immune evasion mechanisms.
- Screening programs for *H.pylori* eradication using *H.pylori* strip test for stool antigen is indicated.
- Further studies focusing on the families of *H. pylori*-positive patients are needed through observations of infection in more than one family member as *H. pylori* infections exhibit intrafamilial clustering.It can help differentiate between genetic susceptibility to infection and environmental factors (e.g., hygiene practices or socio-economic conditions).
- Assessment of noninvasive molecular detection of clarithromycin resistance and detection of other resistant genes.
- Combined assessment of resistance by molecular detection in association with the culture-based sensitivity testing for phenotypic detection of resistance.

Limitations of the study

- Lack of funding and financial constraints limited the ability to detect other resistant genes.
- Budget restrictions confined the study to a single geographic region.
- Budget restrictions also limited the ability to study familial clustering of Helicobacter pylori (*H. pylori*) infections which is a fascinating area of research that has the potential to shed light on key aspects of transmission and resistance.

Conflict of interest

None declared.

Financial disclosure

None declared.

References

1- Diaconu S, Predescu A, Moldoveanu A, Pop CS, Fierbințeanu-Braticevici C. Helicobacter pylori infection: old and new. J Med Life. 2017;10(2):112-117.

2- Borka Balas R, Meliț LE, Mărginean CO. Worldwide Prevalence and Risk Factors of Helicobacter pylori Infection in Children. Children. 2022; 9(9):1359. https://doi.org/10.3390/children9091359.

3- Metwally M, Ragab R, Abdel Hamid HS, Emara N, Elkholy H. Helicobacter pylori Antibiotic Resistance in Egypt: A Single-Center Study. Infect Drug Resist. 2022; 15:5905-5913.http://doi:10.2147/IDR.S386082

4- Ieni A, Barresi V, Rigoli L, Fedele F, Tuccari G, Caruso RA. Morphological and Cellular Features of Innate Immune Reaction in Helicobacter pylori Gastritis: A Brief Review. International Journal of Molecular Sciences. 2016; 17(1):109. https://doi.org/10.3390/ijms17010109

5- Camilo V, Sugiyama T, Touati E. Pathogenesis of Helicobacter pylori infection. Journal Helicobacter 2017; 22(1):12405. https://doi.org/10.1111/hel.12405.

6- Peng X, Song Z, He L. Gastric Juice-Based Real-Time PCR for Tailored Helicobacter Pylori Treatment: A Practical Approach. Int J Med Sci. 2017;14(6):595-601. doi:10.7150/ijms.18996.

7- Lo CC, Lai KH, Peng NJ. Polymerase chain reaction: a sensitive method for detecting Helicobacter pylori infection in bleeding peptic ulcers. World J Gastroenterol. 2005;11(25):3909-3914. doi:10.3748/wjg.v11.i25.3909.

8- Chey WD, Leontiadis GI, Howden CW, Moss SF. ACG Clinical Guideline: Treatment of Helicobacter pylori Infection.

Am J Gastroenterol 2018; 113(7):1102. doi: 10.1038/s41395-018-0132-6.

9- Thung I, Aramin H, Vavinskaya V. Review article: the global emergence of Helicobacter pylori antibiotic resistance. Aliment Pharmacol Ther. 2016; (4):514-533. doi: 10.1111/apt.13497

10- Katelaris P, Hunt R, Bazzoli F. Helicobacter pylori World Gastroenterology Organization Global Guideline. J Clin Gastroenterol. 2023;57(2):111-126. doi:10.1097/MCG.0000000000001719.

11- Liou JM, Chang CY, Sheng WH. Genotypic resistance in Helicobacter pylori strains correlates with susceptibility test and treatment outcomes after levofloxacin- and clarithromycin-based therapies. Antimicrob Agents Chemother. 2011;55(3):1123-1129. doi: 10.1128/AAC.01131-10.

12- Crowe SE. Helicobacter pylori Infection. N Engl J Med. 2019;380(12):1158-1165. doi:10.1056/NEJMcp1710945.

13- Garza-González E, Perez-Perez GI, Maldonado-Garza HJ, Bosques-Padilla FJ. A review of Helicobacter pylori diagnosis, treatment, and methods to detect eradication. World J Gastroenterol. 2014;20(6):1438-1449. doi:10.3748/wjg. v20.i6.1438.

14- Best LM, Takwoingi Y, Siddique S. Non-invasive diagnostic tests for Helicobacter pylori infection. Cochrane Database Syst Rev. 2018;3(3):CD012080. doi: 10.1002/14651858.CD012080.pub2

15- Xiong LJ, Tong Y, Wang Z, Mao M. Detection of clarithromycin-resistant Helicobacter pylori by stool PCR in children: a comprehensive review of literature. Helicobacter. 2013;18(2):89-101. doi:10.1111/hel.12016.

16- Skrebinska S, Daugule I, Santare D. Accuracy of two plasma antibody tests and faecal antigen test for non-invasive detection of H. pylori in middle-aged Caucasian general population sample. Scand J Gastroenterol. 2018;53(7):777-783. doi:10.1080/00365521.2018.1476909

17- Redondo JJ, Keller PM, Zbinden R, Wagner K. A novel RT-PCR for the detection of Helicobacter pylori and identification of clarithromycin resistance mediated by mutations in the 23S rRNA gene. Diagn Microbiol Infect Dis. 2018;90(1):1-6. doi: 10.1016/j.diagmicrobio.2017.09.014.

18- Tran VH, Ha TMT, Le PTQ, Nguyen VN, Phan TN, Paglietti B. Helicobacter pylori 23S rRNA gene mutations associated with clarithromycin resistance in chronic gastritis in Vietnam. J Infect Dev Ctries. 2018;12(7):526-532. Published 2018 Jul 31. doi:10.3855/jidc.10000

19- Sharbatdaran M, Kashifard M, Shefaee S, Siadati S, Jahed B, Asgari S. Comparison of stool antigen test with gastric biopsy for the detection of Helicobacter Pylori infection. Pak J Med Sci. 2013;29(1):68-71. doi:10.12669/pjms.291.2865

20- Gehlot V, Mahant S, Mukhopadhyay AK. Low prevalence of clarithromycin-resistant Helicobacter pylori isolates with A2143G point mutation in the 23S rRNA gene in North India. J Glob Antimicrob Resist. 2016; 6:39-43. doi: 10.1016/j.jgar.2016.02.007

21- Soltan MA, Mansour MA, Zahir TI, Rashed HE, Fahmy YA. Prediction of Helicobacter pylori clarithromycin resistance by detection of point mutations in 23S rRNA gene. Egypt J Med Microbiol. 2018;27(3):13-20 doi: 10.21608/ejmm.2018.285585.

22- Zamani M, Ebrahimtabar F, Zamani V. Systematic review with meta-analysis: the worldwide prevalence of Helicobacter pylori infection. Aliment Pharmacol Ther. 2018;47(7):868-876. doi:10.1111/apt.14561.

23- Hulten KG, Lamberth LB, Kalfus IN, Graham DY. National and Regional US Antibiotic Resistance to Helicobacter pylori: Lessons From a Clinical Trial. Gastroenterology. 2021;161(1):342-344.e1. doi: 10.1053/j.gastro.2021.03.045

24- Ibrahim A, Morais S, Ferro A, Lunet N, Peleteiro B. Sex-differences in the prevalence of Helicobacter pylori infection in pediatric and adult populations: Systematic review and meta-analysis of 244 studies. Dig Liver Dis. 2017;49(7):742-749. doi: 10.1016/j.dld.2017.03.019

25- Bińkowska A, Biernat MM, Łaczmański Ł, Gościniak G. Molecular Patterns of Resistance Among Helicobacter pylori Strains in South-Western Poland. Front Microbiol. 2018; 9:3154. Published 2018 Dec 18. doi:10.3389/fmicb.2018.03154

26- Zaki ME, Sherif DM, Ali MA. Molecular study of primary clarithromycin resistant Helicobacter pylori strains from Egyptian centre. Int.J.Curr.Microbiol.App.Sci.2016; 5: 165-173. doi: http://dx.doi.org/10.20546/ijcmas.2016.501.014

27- Leonardi, M., La Marca, G., Pajola, B. Assessment of real-time PCR for Helicobacter pylori DNA detection in stool with co-infection of intestinal parasites: a comparative study of DNA extraction methods. BMC Microbiol.2020;20(1):1-8. https://doi.org/10.1186/s12866-020-01824-5

28- Metwally M, Ragab R, Abdel Hamid HS, Emara N, Elkholy H. Helicobacter pylori Antibiotic Resistance in Egypt: A Single-Center Study. Infect Drug Resist. 2022; 15:5905-5913. doi:10.2147/IDR.S386082

29- Hussein RA, Al-Ouqaili MTS, Majeed YH. Detection of clarithromycin resistance and 23SrRNA point mutations in clinical isolates of Helicobacter pylori isolates: Phenotypic and molecular methods. Saudi J Biol Sci. 2022;29(1):513-520. doi: 10.1016/j.sjbs.2021.09.024.

30- Nyi KN, Soe AM, Htut ZM (2021): Molecular detection of genotypic clarithromycin-resistant strains and its effect on the eradication rate of concomitant therapy in Helicobacter pylori infection. Gastroenterol Hepatol. 2021; 3(6): 372-378. doi: 10.1002/ygh2.476

31- Kocsmár É, Buzás GM, Szirtes I. Primary and secondary clarithromycin resistance in Helicobacter pylori and mathematical modeling of the role of macrolides. Nat Commun. 2021;12(1):2255. doi:10.1038/s41467-021-22557-7

32- Jaka H, Rhee JA, Östlundh L. The magnitude of antibiotic resistance to Helicobacter pylori in Africa and identified mutations which confer resistance to antibiotics: systematic review and meta-analysis. BMC Infect Dis. 2018;18(1): 193.doi:10.1186/s12879-018-3099-4

33- White JR, Sami SS, Reddiar D. Narrow band imaging and serology in the assessment of premalignant gastric pathology. Scand J Gastroenterol.2018;53(12):1611-1618. doi:10.1080/00365521.2018.1542455

34- Mégraud F, Graham DY, Howden CW. Rates of Antimicrobial Resistance in Helicobacter pylori Isolates From Clinical Trial Patients Across the US and Europe. Am

J Gastroenterol. 2023;118(2):269-275. doi:10.14309/ajg.0000000000002045

35- Abadi AT, Taghvaei T, Ghasemzadeh A, Mobarez AM. High frequency of A2143G mutation in clarithromycin-resistant Helicobacter pylori isolates recovered from dyspeptic patients in Iran. Saudi J Gastroenterol. 2011;17(6):396-399. doi:10.4103/1319-3767.87181

36- Mégraud F. H pylori antibiotic resistance: prevalence, importance, and advances in testing. Gut. 2004;53(9):1374-1384. doi:10.1136/gut.2003.022111

37- Klesiewicz K, Nowak P, Karczewska E. PCR-RFLP detection of point mutations A2143G and A2142G in 23S rRNA gene conferring resistance to clarithromycin in Helicobacter pylori strains. Acta Biochim Pol. 2014;61(2):311-315.

38- Bińkowska A, Biernat MM, Łaczmański Ł, Gościniak G. Molecular Patterns of

39- Resistance Among Helicobacter pylori Strains in South-Western Poland. Front Microbiol. 2018; 9:3154. doi:10.3389/fmicb.2018.03154

40- Eghbali Z, Mojtahedi A, Moien Ansar M, Fakhrieh Asl S, Aminian K. Detection of 23SrRNA Mutations Strongly Related to Clarithromycin Resistance in Helicobacter pylori Strains Isolated from Patients in the North of Iran. Jundishapur J Microbiol. 2016;9(2): e29694. Published 2016 Feb 15. doi:10.5812/jjm.29694

41- Abdollahi H, Savari M, Zahedi MJ, Moghadam SD, Hayatbakhsh Abasi M. Detection of A2142C, A2142G, and A2143G Mutations in 23s rRNA Gene Conferring Resistance to Clarithromycin among Helicobacter pylori Isolates in Kerman, Iran. Iran J Med Sci. 2011;36(2):104-110.

42- Park CG, Kim S, Lee EJ, Jeon HS, Han S. Clinical relevance of point mutations in the 23S rRNA gene in Helicobacter pylori eradication: A prospective, observational study. Medicine (Baltimore). 2018;97(33): e11835. doi:10.1097/MD.0000000000011835

43- Ji Z, Han F, Meng F, Tu M, Yang N, Zhang J. The Association of Age and Antibiotic Resistance of Helicobacter Pylori: A Study in Jiaxing City, Zhejiang Province, China. Medicine (Baltimore). 2016;95(8):e2831. doi:10.1097/MD.0000000000002831.

44- Gisbert JP. Empirical or susceptibility-guided treatment for Helicobacter pylori infection? A comprehensive review. Therap Adv in Gastroenterol. 2020 ;Nov; 13:1756284820968736

Mohamed MAS, Abdelhalim RM, Kassem NN, Kamel EM, Saber SM, Elsayed EY, Rashad SSM. Molecular detection of clarithromycin-resistant *Helicobacter pylori* in stool samples using real-time PCR. Microbes Infect Dis 2025; 6(3): 3051-3064.