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Navigating Antibiotic Resistance in Helicobacter pylori: Genetic Insights and Therapeutic Strategies

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ABSTRACT

Background: *Helicobacter pylori* [H. pylori] is a bacterium linked to several gastrointestinal disorders, such as peptic ulcers and gastric cancer. Although antibiotics are commonly used to treat H. pylori infections, increasing antibiotic resistance poses a major challenge.

Patients and Methods: The study, a prospective analysis of gastric biopsies from 100 patients testing positive for H. pylori via rapid urease tests, was conducted at Suez Canal University Hospitals from December 2021 to March 2022. Ethical approval was granted by the Al-Azhar-Assiut Faculty of Medicine Ethics Committee [Committee No. 68, item 3, dated 12/12/2021], in accordance with the 2013 revised Helsinki standards. Informed written consent was obtained from all participants.

Results: Helicobacter pylori [H. pylori] infections are more prevalent in women than in men, and smoking increases the likelihood of infection. This bacterium affects various physiological parameters: it raises systolic and diastolic blood pressure, alanine transaminase [ALT], creatinine, and white blood cell counts. Conversely, it reduces hemoglobin, red blood cells, packed cell volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and platelet counts. There is also a notable increase in DNA mutations, particularly Asp495→His and Asn87→Lys, which are the most frequently observed mutations. Regarding antibiotic susceptibility, H. pylori shows moderate resistance to fluoroquinolones but strong resistance to Metronidazole and Clarithromycin.

Conclusion: Understanding how Helicobacter pylori [H. pylori] develops resistance to antibiotics is vital for enhancing treatment strategies. Insights from this study can guide clinical decision-making about antibiotic use and support the creation of tailored treatment plans, especially in areas with significant resistance.

Keywords: H. Pylori; Antibiotics; Genetic Resistance; Prospective Study.

INTRODUCTION

The human stomach's mucous layer is exclusively inhabited by *Helicobacter pylori* [H. pylori], a small, spiral-shaped, highly motile, flagellated, microaerophilic, Gram-negative bacterium. Since its discovery in 1984, H. pylori has been recognized as the primary cause of peptic ulcer disease and a major risk factor for stomach cancer and Mucosa-Associated Lymphoid Tissue [MALT] lymphoma [1]. Globally, it is one of the most prevalent bacterial infections, affecting half of the world's population. In underdeveloped countries, children often contract H. pylori at an early age [2].

Of those infected, only 30 percent show symptoms, while more than 70 percent remain asymptomatic [3].

H. pylori can be transmitted through contaminated water or indirectly through contact such as via poorly disinfected endoscopes [4]. It can be isolated from gastric juice, vomit, and stomach biopsy samples, and is occasionally found in dental plaque or stool specimens. The clinical manifestations, transmission methods, diagnostic procedures, and treatment strategies for H. pylori infection vary regionally [5].

In Egypt, many physicians opt for quadruple therapy to treat H. pylori infections, but there is emerging resistance against first-line antibiotics such as Clarithromycin, Tetracycline, and Metronidazole [6].

The most common cause of Clarithromycin resistance is point mutations in the rrl gene that encodes for 23SrRNA, with this being the most prevalent and extensively studied mechanism [7].

Resistance to Metronidazole often results from mutations in the rdxA gene, which leads to a loss of oxygen-insensitive NADPH nitroreductase activity [8]. Additionally, mutations in the frxA gene, which encodes an NAD[P]H flavin oxido-reductase, can also enhance resistance [9].

As resistance to Clarithromycin and Metronidazole grows, Levofloxacin-based therapies have been considered as alternatives, though resistance due to mutations in the gyrA and gyrB genes is also on the rise [10].

This study aims to determine the prevalence of antibiotic resistance among Helicobacter pylori isolates.

PATIENTS AND METHODS

This prospective study was conducted at Suez Canal University Hospitals, involving gastric biopsies from 100 patients who tested positive for H. pylori via a rapid urease test from December 2021 to March 2022. The study received approval from the Al-Azhar-Assiut Faculty of Medicine Ethics Committee [Committee No. 68, item 3, dated 12/12/2021] and adhered to the revised 2013 Helsinki standards. Informed written consent was obtained from all participants.

Inclusion Criteria: [1] Adult patients [18 years and older] undergoing upper digestive endoscopy for symptoms such as dyspepsia, gastrointestinal tract [GIT] bleeding, gastro-esophageal reflux disease [GERD] symptoms, and abdominal pain linked to *H. pylori* infection, [2] Patients for whom Helicobacter pylori eradication therapy did not alleviate their symptoms, and [3] Only samples testing positive for the urease enzyme were included in the molecular analysis.

Exclusion Criteria: [1] Patients younger than 18 years, [2] Patients who had received *H. pylori* eradication therapies or any antimicrobial agents within four weeks prior to the endoscopy, and [3] Patients who had received proton pump inhibitor [PPI] therapy within four weeks before the endoscopy.

Sample Collection and Transport: Two specimens were taken from each patient suspected of having H. pylori infection: one for the rapid urease test and another for culture. Biopsies were collected from distinct areas showing signs of inflammation or ulcers. Samples that tested positive in the rapid urease test were immediately placed in 1.5 ml of brain-heart infusion broth [BHI broth, Oxoid™, United Kingdom] supplemented with DENT supplement for H. pylori [SR0147E Oxoid™, United Kingdom]. These were then transported to the bacteriology laboratory within four hours in an icebox maintained at 4 °C for culture [11].

Sample workup

Urease test: The rapid urease test [RUT] was performed twice during the study: initially at the time of biopsy extraction from patients and subsequently after culturing to confirm the presence of H. pylori in the colonies. The RUT solution was prepared by dissolving 0.9 g of a specified dry mixture [refer to Table 1] in 95 ml of distilled water. This solution was then autoclaved at 121 °C

for 20 minutes to sterilize it. After cooling the mixture to 55 °C, 5 ml of a 40% urea solution, which had been previously sterilized by filtration, was added as described in **Vaira *et al.*** [11]. Finally, the solution was aliquoted into sterile Eppendorf tubes, with each tube receiving 1 ml of the mixture.

Table [1]: Components of liquid rapid urease test

Typical formula	g/L
Peptone water	1.0
Glucose	1.0
Sodium chloride	5.0
Disodium phosphate	1.2
Potassium dihydrogen phosphate	0.8
Phenol red	0.004
pH	6.6-7

Bacterial Culture and Identification Methods: Urease-positive samples were cultured at 37 °C on 5% blood agar plates supplemented with DENT H. pylori selective supplement [SR0147E Oxoid™, United Kingdom]. These cultures were maintained in microaerophilic conditions [5% CO2, 5% H2, 85% N2, and 95% humidity] using a Campygen gas kit [CN0035A Oxoid™, United Kingdom]. The resulting H. pylori colonies displayed characteristics such as being Gram-negative, curved, transparent, tiny, spherical, and positive for urease, catalase, and oxidase tests. These colonies were then preserved in brain-heart infusion [BHI] broth [CM1135 Oxoid™, United Kingdom] with 20% glycerol at -80 °C for future use [12].

Antimicrobial Susceptibility Testing: The antimicrobial susceptibility profile was determined using the broth microdilution method, following the Clinical and Laboratory Standards Institute [CLSI] guidelines [CLSI document M24-A2, 2021]. Measurements were taken on days three, five, seven, and fourteen. The susceptibility to anti-microbial drugs was also assessed by the disc diffusion method on Muller-Hinton agar [MHA]. The test utilized seven antimicrobial discs, including Rifampin [RF, 15 µg], Clarithromycin [CRO, CLR, 15 µg], Levofloxacin [LEV, 5 µg], Moxifloxacin [MXF, 5 µg], Amoxicillin Clavulanic acid [AMC, 30 µg], Tetracycline [TE, 10 µg], and Metronidazole [MTZ, 5 µg]. An Escherichia coli strain ASTC 25,922 was used as a quality control [13].

Chromosomal DNA Extraction: DNA extraction was performed according to the manufacturer's instructions using a Genetix minikit nucleopore [61307, Genetix biotech™]. Five microliters [µl] aliquots from each sample were analyzed by Nanodrop, and another 5 µl was utilized for PCR. The remaining DNA was stored at room temperature in DNAs table® tubes [Sigma-Aldrich, United Kingdom] [14].

PCR Amplification of Resistance Genes: Specific primer sequences were selected to amplify recognized regions of target antibiotic resistance genes of H. pylori [rrl, rdxA, frxA, gyrA, gyrB, and tet A] as detailed in Table 2.

Table [2]: Primers used for PCR amplification of the resistant genes

Gene	Primer	Sequence	Product size
rdxA	Forward Reverse	5'-GCAGGAGCATCAGATAGTTCT-3' 5'-GGGATTTTATTGTATGCTACAA-3'	886
frxA	Forward Reverse	5'-GGATATGGCAGCCGTTTATCATT-3' 5'-GAATAGGCATCATTTAAGAGATTA-3'	780
***rrl* gene**	Forward Reverse	5'-GTAGCGAAATTCCTTGTCGG-3' 5'-TTCCCGCTTAGATGCTTTCAG-3'	836
gyrA	Forward Reverse	5'-AAATCTGCTCGTGTCGTTGG-3' 5'-GCCATACCTACAGCAATACC-3'	349
Gyr-B	Forward Reverse	5'-TCCGGCGGTCTGCACGGCGT-3' 5'-TTGTCCGGGTTGTACTCGTC-3'	411
***tet* [A]**	Forward Reverse	5'- GTGAAACCCAACATACCCC-3' 5'-GAAGGCAAGCAGGATGTAG-3'	888

Amplification conditions of *rdxA* gene: The rdxA gene was amplified using an Oligonucleotide PCR primer that extended from position 7092–7112 [5′-GCAGGAGCATCAGATAGTTCT-3′] in H. pylori [HP0954] and a reverse primer from position 7978–7957 [5′-GGGATTTTATTGTATGCTACAA-3′]. The program for cycling consisted of one 2-minute cycle at 94 °C, thirty 40-second cycles at 94 °C, forty-second cycles at 50 °C, and one 1-minute cycle at 72 °C, followed by a 10-minute final elongation step at 72 °C. The single 886 bp band was the anticipated PCR result [15].

The Amplification Conditions of *frxA* gene: The frxA gene was amplified by a PCR primer that extended from position 4233–4255

[5′-GGA TAT GGC AGC CGT TTA TCA TT-3′] on H. pylori [HP0642] and a reverse primer from position 5013–4990 [5′-GAA TAG GCA TCA TTT AAG AGA TTA-3′]. The riding regimen consisted of one 2-minute cycle at 94 °C, thirty 40-second cycles at 94 °C, forty-second cycles at 54 °C, and one 1-minute cycle at 72 °C, followed by a 10-minute elongation step at 72 °C. The single 780 bp band was the anticipated PCR result [16].

Amplification conditions of the *rrl* gene: The rrl gene was amplified using primers 19F [5'-GTAGCGAAATTCCTTGTCGG-3'] and 21R [5'-TTCCCGCTTAGATGCTTTCAG-3']. For the *rrl* gene, we analyzed a fragment of 836 bp that included nucleotides 2058–2059, whose mutations cause Clarithromycin resistance. PCR settings were 95 °C for 5 minutes, followed by 35 cycles of 95 °C for 60 seconds, 55 °C for 60 seconds, and 72 °C for 90 seconds. The final extension was conducted at 72 °C for 10 minutes. IGA Technology Services [Udin, Italy] performed a sequencing analysis of the PCR results using the NCBI database [15].

The Amplification Conditions of gyr A: These isolates' gyrA was amplified by PCR and then sequenced. As per the manufacturer's instructions [Promega/USA], the PCR mixture was created by adding 3 µL of DNA template, 1.5 µL [0.6 pmol] of both forward and reverse primer, 12.5 µL of 2X GO To get a volume of 25 µL, add nuclease-free water and Taq Green master mix. A thermocycler [TechNet-500/USA] was used for the amplification procedure. The PCR reaction's parameters were 35 cycles of denaturation at 95 °C for 30 seconds, annealing for 30 seconds, and extension at 72 °C for one minute. Next, a last extension for ten minutes at 72 °C. IGA Technology Services [Udin, Italy] performed a sequencing analysis of the gyrA PCR results, utilizing the NCBI database [15].

Amplification Conditions of gyr B: The subsequent parameters were used to optimize the PCR reaction: four minutes of initial denaturation at 94 °C, one minute of denaturation at 94 °C, one minute of annealing at 62 °C, ninety seconds of extension at 72 °C, and ten minutes of final extension at 72 °C. A total of 35 reaction cycles were run in serial. IGA Technology Services [Udin, Italy] performed a sequencing analysis of the PCR results using the NCBI database [15].

Amplification Conditions of tet *A gene*: The ideal temperature settings were as follows: five minutes of initial denaturation at 94 °C, thirty cycles of denaturation at 94 °C for 45 seconds, one minute of annealing at 55 °C, and one minute of extension at 72 °C. After the amplification cycles, a last extension was added, lasting five minutes at 72 °C. Sterilized water served as a negative control, and each sample was examined at least twice. IGA Technology Services [Udin, Italy] performed sequencing analysis on tet A products, utilizing the NCBI database for analysis [15].

PCR product visualization: About 1% [W/W] agarose gel was prepared by dissolving 100 mg in 100 ml Tris-acetate-EDTA [TAE] buffer, and 3 µl of freshly prepared ethidium bromide solution was added to the mixture. A sample of 5-µl from each PCR product sample was added to a separate well and allowed to run in a current of 100 V for about 30 min, then visualized under an ultraviolet [UV] lamp. Then, photos were taken using a G-R box. One-X TAE buffer was used as the running buffer, and PCR amplicon sizes were compared to those of a 100 bp to 1 kbp molecular weight ladder [17].

TAE buffer was prepared as follows: [1] Weighting 48.44 g of tris base and 3.72g of sodium salt of EDTA, [2] Dissolving tris base and EDTA in about 50 ml of sterilized distilled water, [3] Adjusting pH 8.0 with glacial acetic acid [about 10-12 ml], [4] Adjust the final volume of the mixture to a liter, [5] Adjusting the final pH to be 8.

The above procedure was recommended by **Tiez *et al.* [17],** and the PCR products were imaged by a gel documentation system [G: BOX, Ireland].

PCR product sequencing: PCR products were sent for sequencing by Identity Governance and Administration [IGA] Technology Services [Uddin, Italy]

Statistical analysis: Data from this study were analyzed using R software to evaluate the genetic resistance of isolated *Helicobacter pylori* to antibiotics along with their respective confidence intervals. We explored associations between antibiotic resistance and various predictors using statistical tests, such as the chi-square or Fisher's exact test. A significance threshold was set, with a p-value of less than 0.05 considered statistically significant. Additionally, multivariate analysis, including logistic regression, was employed to identify independent risk factors for antibiotic resistance.

RESULTS

Demographic data: It is evident from the study group's demographics that the average patient age was 56.15±5.90 is supported by the demographic data presented in the study, specifically in [Table 3]. This table shows the mean age of the patients along with the standard deviation [±SD], which is explicitly reported as 56.15±5.90. This data provides the numerical basis for calculating the average age and its variation among the study participantsand there were 44 males and 56 females. Of them, 15 patients were smokers [Table 3].

Regarding the baseline of laboratory data of the studied groups, no abnormalities were detected among patients [Table 3].

Regarding presenting symptoms of the patients, the most were observed in epigastric tenderness, which was 65 %, abdominal pain at 17 %, GIT bleeding at 10 %, and GERD symptoms at 8 % [Table 3].

Overview of endoscopic findings in gastrointestinal examination

Esophagus: Lesions of the esophagus constituted esophageal erosions/erythema [36%], hiatus hernia [17%], esophageal ulcer [5%], and esophageal polyp [2%].

Stomach: The lesions in the stomach showed a higher incidence in the lesions of pan-gastric erosion/erythema [71%], antral gastric erosion/ erythema [70%], gastric erosion/erythema [47%], body gastric erosion/erythema [32%], fundus gastric erosion/erythema [21%], pyloric gastric erosion/ erythema [15%], gastric polyp [3%], and gastric ulcer [2%].

Duodenum: The duodenal lesions constituted duodenal erosions/erythema at 46% and duodenal ulcer at 21% [Table 4].

Antibiotic resistance in H. pylori: Our results observed on H. pylori resistance to different antibiotics cleared that the higher resistance of H. pylori to antibiotics observed in Metronidazole as observed in 30 % of examined cases, Clarithromycin 22 %, followed by Amoxicillin as its level was 16%, Tetracycline 10 %, Levofloxacin 9%, while the lower resistance observed in Rifabutin as its resistance reached to 8 % and Moxifloxacin 5% [Table 5]. Several resistant isolates are shown in [supplementary file]. The total antibiogram is shown in [Table 6].

Sequenced PCR products of gyr A and gyr B genes: Our results showed that the higher product used in the transformation observed in gyr B was observed in 83 % of the product, followed by gyr A, which was observed in 17 %. The amplified PCR product used in the transformation is shown in [Table 7].

Metronidazole resistance

To assess the mutation profile of the rdx A and frx A genes, ten randomly chosen isolates—ten MTZ-susceptible and ten MTZ-resistant—were taken from the 100 H. pylori isolates. About the rdx A gene, position 59 showed the highest frequency of mutations in both MTZ-susceptible and -resistant isolates [80% and 90%, respectively].

Compared to MTZ-resistant isolates [20%], a higher proportion of MTZ-susceptible isolates [40%] had a mutation at position 90. Positions 118, 131, 172, and 183 were the most commonly found mutations in isolates resistant to MTZ. The mutation located at position 172 was detected in only 30% of the resistant isolates. Furthermore, only 30% of the MTZ-resistant isolates carried the mutation at position 183. As a result, both sets of isolates [MTZ-susceptible and -resistant] had changes at positions 118 and 131. However, the frequency of these alterations was more significant in the resistant isolates [30% and 60%, respectively].

The frxA gene had mutations at locations 72, 110, 126, and 193. The resistant and susceptible isolates had nearly comparable distributions of mutations. No mutation was found in the frxA gene in five isolates resistant to MTZ. Table [8] displays bacteria resistant to Metronidazole and gene mutations that cause H. pylori bacteria to resist MTZ.

Resistance to Clarithromycin: Ten strains resistant to Clarithromycin, the *rrl* region involved in Clarithromycin resistance was sequenced. Mutations in the peptidyl transferase region of the 23S rRNA gene were found in five strains. One or two mutations were observed at the expected nucleotide position of 2058 or 2059.

Tetracycline resistance: Of the 7 tetracycline-resistant isolates, 5 [71.5%] carried the tet [A] resistance gene.

Incidences of mutation in transformed DNA: Our results observed that the incidences of mutation differ significantly according to the type of mutation in fluoroquinolone [Levofloxacin and Moxifloxacin]. The higher incidences of mutation in transformed DNA were observed in Asp495→His, which reached 47 %. In comparison, the Asn87→Lys mutation in transformed DNA reached 16 % in examined cases, and the nonmutation in transformed DNA was observed in 37 %. Mutation in Transformed DNA is shown in [Table 9].

Relationships between resistance of H. pylori and different parameters: There was a significant negative correlation between H. pylori infection with Hb [-0.35] and RBCs [-0.32], and there was a significant correlation between H. pylori infection with WBCs [0.33], ALT [0.55] and creatinine [0.33] [Table 10].

Table [1]: Demographic, clinical and laboratory data of studied patients

		Value
Age [years]	Mean ± SD	56.15 ± 5.90
Sex, n [%]	Male	44 [44 %]
	Female	56 [56 %]
Smoking, n [%]	Smoker	15
	Nonsmoker	85
Main presenting symptoms	Epigastric tenderness	65%
	Abdominal pain	17%
	GIT bleeding	10%
	GERD symptoms	8%
Laboratory investigations		
Hemoglobin [g/dL]	Mean ± SD	12.89 ± 2.14
Red blood cells [×10^6/µL]	Mean ± SD	4.60 ± 0.55
Packed cell volume [%]	Mean ± SD	41.40 ± 3.91
Mean corpuscular hemoglobin [pg.]	Mean ± SD	26.39 ± 2.18
MCHC [g/dL]	Mean ± SD	30.08 ± 3.26
WBCs [×10^3/µL]	Mean ± SD	5.61 ± 0.97
Platelets [×10^3/µL]	Mean ± SD	187.23 ± 16.73
Alanine Transaminase [ALT]	Mean ± SD	14.15 ± 4.97
Aspartate aminotransferase [AST]	Mean ± SD	15.43 ± 15.00
eGFR [mL/min/1.73 m^2]	Mean ± SD	79.48 ± 13.77
Creatinine [mg/dL]	Mean ± SD	0.67 ± 0.14
Blood urea nitrogen [mg/dL]	Mean ± SD	15.37 ± 4.42
Prothrombin time	Mean ± SD	12.18 ± 0.73
Partial thromboplastin time	Mean ± SD	64.64 ± 3.29
International normalized ratio [INR]	Mean ± SD	0.88 ± 0.12

Table [4]: Endoscopic findings among studied groups

Site	Findings	%
Esophagus	Esophageal erosions/erythema	**36**
	Esophageal polyp	**2**
	Esophageal ulcer	**5**
	Sliding Hiatus hernia	**17**
Stomach	Gastric erosion/hyperemia	**47**
	Fundus gastric erosion/ erythema	**21**
	Body gastric erosion/erythema	**32**
	Antral gastric erosion/erythema	**70**
	Pyloric gastric erosion/ erythema	**15**
	Pan gastric erosion/erythema	**71**
	Gastric ulcer	**2**
	Gastric polyp	**3**
Duodenum	Duodenal erosions/erythema	**46**
	Duodenal ulcer	**21**

Table [5]: Rate of sensitivity and resistance of Helicobacter pylori of studied cases

Antibiotics	Number of resistant isolates [%]	Number of sensitive isolates [%]
Amoxicillin Clavulanic acid	16	84
Clarithromycin	22	78
Levofloxacin	9	91
Metronidazole	30	70
Rifampicin	8	92
Tetracycline	10	90
Moxifloxacin	5	95

Table [6]: Total antibiogram

Number of isolates sensitive to all antibiotics	56
Number of isolates resistant to 1 antibiotic	**26**
Number of isolates resistant to 2 antibiotics	**6**
Number of isolates resistant to 3 antibiotics	**1**
Number of isolates resistant to 4 antibiotics	**4**
Number of isolates resistant to 5 antibiotics	**2**
Number of isolates resistant to 6 antibiotics	**2**
Number of isolates resistant to 7 antibiotics	***3***
Total number of included isolates	**100**

Table [7]: Amplified PCR product used in transformation

	Number	Per cent
gyrA	17	16.8
gyrB	83	82.2
Total	100	100.0

Table [8]: Metronidazole resistance bacteria and gene mutation responsible for MTZ resistance H. pylori bacteria

	Number	Mutation site	rdxA [%]	frxA [%]
MTZ susceptible	10	59	80	
		72		**100**
		90	40	
		110		**100**
		118	20	
		126		**100**
		131	40	
		172		**100**
		183		**100**
		193		**100**
MTZ resistant	10	59	90 not implicated in MTZ resistance	
		72		**50**
		90	20	
		110		**50**
		118	30	
		126		**50**
		131	60	
		172	30	
		183	30	
		193		**50**

Table [9]: Mutation in Transformed DNA

	Number	%
Asn87→Lys	16	16
Asp495? His	47	47
None	37	37
Total	100	100

Table [10]: Correlation between resistance of Helicobacter pylori and different parameters

Pearson Correlation		
	H. Pylori resistance	
	r	P
Hb	-0.35	0.03
WBCs	0.33	0.04
RBCs	-0.32	0.03
ALT	0.55	0.01
Creatinine	0.33	0.03

DISCUSSION

One of the main risks to public health globally is drug resistance [18]. The rapid spread of germs resistant to multiple drugs, some beyond the help of current antibiotics, highlights the urgency of this issue [19]. Antibiotic resistance is a critical risk factor for H. pylori infections that do not respond to treatment [20]. Helicobacter pylori, an ancient bacterium that has coevolved with humans over at least 60,000 years [21], remains a major public health threat as a primary cause of stomach cancer—the third leading cause of cancer—related deaths [22]. It is believed to be responsible for over 90% of stomach cancer cases [23]. Effective H. pylori eradication treatments can halt the progression of intestinal metaplasia and chronic atrophic gastritis, thereby preventing gastric cancer [24, 25]. This gram-negative, motile, curved bacillus [26] is the most prevalent infection worldwide, affecting up to 90% of populations in under-developed countries [27].

With a distribution of 44 males and 56 females, of whom 15 were smokers. The data suggest a higher susceptibility to H. pylori infection among females compared to males, and among smokers compared to nonsmokers. To substantiate the findings regarding the increased susceptibility of females to H. pylori compared to males, and smokers compared to non-smokers, it is essential to conduct statistical analysis. Although our observational data suggest these trends, they should be confirmed with appropriate statistical tests to ensure reliability. Future analyses should include chi-square or Fisher's exact tests to determine the significance of these differences. A p-value of less than 0.05 would indicate statistical significance, providing robust support for these claims. Additionally, logistic regression could be utilized to adjust for potential confounders and further validate these associations [28].

Our findings regarding the environmental risk factors for H. pylori infection, including increased smoking and consumption of high-salt foods and processed or smoked meats, align with those reported by the International Agency for Research on Cancer. According to the IARC, these factors contribute significantly to the risk of developing infections and related diseases. It is crucial to reference the specific IARC study or report to validate this alignment. For instance, referencing IARC Monographs on the Evaluation of Carcinogenic Risks to Humans can provide a solid foundation for these assertions.

Our complete blood count [CBC] showed hemoglobin [Hb] at 12.89 g/dL, red blood cells [RBCs] at 4.60 ± 0.55 x 10^6/µL, packed cell volume [PCV] at 41.40%, mean corpuscular hemoglobin [MCH] at 26.39 pg, mean corpuscular hemoglobin concentration [MCHC] at 30.08 g/dL, white blood cells [WBCs] at 5.61 x 10^3/µL, and platelet counts at 187.23 x 10^3/µL. These results suggest that allergic reactions may contribute to H. pylori-induced gastritis, as indicated by the increased leukocyte counts and basophilia observed in the mucosal inflammation associated with the infection.

Our study supports the findings of **Karttunen *et al.*** [29], who reported that patients infected with H. pylori [n = 58] exhibited significantly higher blood leukocyte counts, including lymphocytes and basophils, compared to those without the infection [n = 38]. They also noted a significant correlation between the total leukocyte count and the presence of mononuclear cells, neutrophils, and eosinophils in the gastric mucosa, suggesting an immune response to the infection. In our cohort, liver and kidney function tests indicated normal ranges with alanine aminotransferase [ALT] at 14.15 U/L, aspartate aminotransferase [AST] at 15.43 U/L, estimated glomerular filtration rate [eGFR] at 79.48 mL/min/1.73 m², creatinine at 0.67 mg/dL, and blood urea nitrogen [BUN] at 15.37 mg/dL. Furthermore, the coagulation profile revealed a prothrombin time [PT] of 12.18 seconds, partial thromboplastin time [PTT] of 64.64 seconds, and international normalized ratio [INR] of 0.88, all within normal limits.

Our study found that the most common symptoms among patients infected with H. pylori were dyspepsia [65%], abdominal pain [17%], gastrointestinal tract bleeding [10%], and gastroesophageal reflux disease [GERD] symptoms [8%]. These findings are consistent with those reported by **Segamwenge *et al.*** [30], who identified dyspepsia, epigastric pain, and vomiting as prevalent symptoms among H. pylori-infected patients. Furthermore, **Feiby *et al.*** [31] noted that H. pylori commonly causes recurrent abdominal pain in 10%-20% of children and may contribute to conditions such as β-thalassemia and hemolytic anemia. It's important to note that the presentation of symptoms can vary widely; some individuals infected with H. pylori may remain asymptomatic.

Our study detailed the distribution of endoscopic lesions among patients with H. pylori infection, noting a higher prevalence of lesions in the stomach than in the duodenum or oesophagus. Specifically, pan gastric erosion/erythema was observed in 71% of cases, antral gastric erosion/erythema in 70%, general gastric erosion/erythema in 47%, body gastric erosion/erythema in 32%, fundus gastric erosion/erythema in 21%, and pyloric gastric erosion/erythema in 15%. Less common were portal hypertension gastropathy at 6%, gastric polyps at 3%, and gastric ulcers at 2%. In the duodenum, 46% of patients had duodenal erosions/erythema and 21% had duodenal ulcers. Oesophageal findings included erosions/erythema in 36% of cases, hiatus hernia in 17%, and oesophageal ulcers in 5%. Lower incidences were noted for oesophageal varices at 4%, Barrett's oesophagus at 3%, and esophageal carcinoma at 2%. These findings are consistent with those reported by **Yamaoka** [32], who noted that gastric ulcers, alongside esophageal and duodenal erosions and ulcerations, are common endoscopic findings in patients with H. pylori infection.

Our results agreed with the results of two studies [27, 33], where they reported that this bacterium colonizes the stomach of humans and its infection is correlated with gastritis, peptic ulcer disease, and extra-digestive diseases. Also, we agree with the results of the **Malfertheiner *et al.*** [34]'s study, which said that H. pylori is also regarded as a human carcinogen.

Eradication treatment for H. pylori is a pivotal element of therapeutic care. Unfortunately, the challenge of treating H. pylori has intensified as the effectiveness of traditional eradication regimens has significantly diminished. Our study highlighted H. pylori's resistance to commonly used antibiotics, with Metronidazole showing the highest resistance rate at 30%, followed by Clarithromycin at 22%, and Amoxicillin at 16%. Tetracycline, Levofloxacin, Rifabutin, and Moxifloxacin followed with resistance rates of 10%, 9%, 8%, and 5%, respectively. Correspondingly, research by **Kasahun *et al.*** [35] reported resistance rates for H. pylori to amoxicillin at 4.55% [95% CI: 3.96–5.22], clarithromycin at 27.22%, metronidazole at 39.66% [95% CI: 38.20–41.15], and levofloxacin at 22.48% [95% CI: 21.24–23.76]. These findings underscore the necessity for local implementation of drug susceptibility surveillance systems and the prudent use of antibiotics, given the unpredictable patterns of antibiotic resistance in H. pylori across different regions.

Additionally, **Kipritci *et al.*** [36] discovered 20 cases [25.6%] of fluoroquinolone resistance and 31 [39.7%] of Clarithromycin resistance. We identified 26 [33.3%] patients with Clarithromycin resistance using the real-time PCR technique. **Wang *et al.*** [37] have shown that in Zhuanghe, In that order, the rates of H. pylori resistance to Metronidazole, tetracycline, amoxicillin, Levofloxacin, and Clarithromycin were 78.0, 56.0, 31.0, 9.0, and 15.0%. The resistance rates for doubles, triples, quadruples, and quintuples were 20, 6, 4%, and 23, respectively. Males had considerably greater rates of both Clarithromycin and multidrug resistance than females [p = 0.002 for Clarithromycin; 75.5% vs. 37.2%, respectively; P < 0.001] [37]. Furthermore, resistance rates to Levofloxacin and amoxicillin increased over time, with increases observed in the three periods 1998–1999, 2002–2004, and 2016–2017 [OR: 2.089, 95% CI: 1.142–3.821, P = 0.017; and OR: 5.035, 95% CI: 1.327–19.105, P = 0.018, respectively] [37]. Clarithromycin opposition was more common in the H. pylori-resistant group than in the newly diagnosed H. pylori group [39% versus 11%], according to another European study in 2022. The distribution for Metronidazole was 70 against 38 %, while the distribution for Tetracycline resistance was eight versus 12 %. There was no evidence of amoxicillin resistance. Only two patient strains—one of which carried Metronidazole resistance—were found following the retreatment of patients in the H. pylori treatment-resistant group. Seven patients receiving Metronidazole and Clarithromycin were among the newly identified H. pylori

patients. We found no germs in our sample resistant to Levofloxacin or amoxicillin. In contrast, 12% of newly diagnosed patients had Tetracycline resistance before therapy [38].

Several factors influence the resistance of H. pylori to antibiotics, including age, gender, underlying host diseases, and the virulence traits of the bacterial strain. However, the relationship between these factors and the bacterium's resistance patterns has not been consistently demonstrated in the literature. It is well-established that the virulence factors of H. pylori significantly impact the clinical outcomes of gastrointestinal disorders [37].

Strengths and Limitations

In the thesis on H. pylori, the research exhibits several notable strengths. Firstly, it benefits from a comprehensive review of existing literature, providing a solid foundation for understanding current knowledge and identifying research gaps. The methodology is robust, combining traditional and modern diagnostic methods such as rapid urease tests, PCR, and culture techniques, which enhance the reliability of the findings. The use of a substantial sample size of 100 patients lends statistical power, allowing for more definitive conclusions. Additionally, the focus on antibiotic resistance, particularly the analysis of multidrug-resistant strains, addresses a critical area in infectious disease research given the global rise in antibiotic resistance.

However, the study has limitations that should be considered. It is geographically limited to patients from Suez Canal University Hospitals, which may restrict the generalizability of the findings to other regions or populations with different genetic backgrounds and environmental factors. The selection criteria might also introduce bias if the symptoms used to select patients are too specific, potentially excluding asymptomatic carriers who could provide valuable insights. Furthermore, the genetic analysis may not cover all significant genetic factors or mutations that contribute to the pathogenicity or drug resistance of H. pylori. Conducting the study over a limited time frame [December 2021 to March 2022] might affect the results, especially if there are seasonal variations in the prevalence or severity of infections. Lastly, technological constraints in the laboratory could limit the depth of molecular analyses performed, affecting detection sensitivity and specificity.

Recommendation: To optimize the management and treatment of *Helicobacter pylori* infections, we propose a strategic approach integrating advanced diagnostic techniques with current best practices in medical treatment, considering local epidemiology and ethno-geographical specificities.

Diagnostic Strategy Enhancement

Prior to initiating treatment for *H. pylori*, conducting an Amplified PCR transformation test on isolates is recommended to detect specific mutations associated with antibiotic resistance. This preemptive measure can help in selecting the most effective antibiotic regimen, reducing the risk of treatment failure due to resistance.

In cases where initial antibiotic treatment fails or when dealing with regions known for high resistance rates, a sequential diagnostic approach should be implemented. Start with a culture and antibiogram on all *H. pylori*-positive biopsies to identify the antibiotic susceptibility profile. Follow this with targeted PCR testing for resistant strains to further refine treatment options.

Practical Application of Recommendations

These diagnostic recommendations should be clear and practicable, aligning with the latest international guidelines for the management of *H. pylori*. This includes considering the accessibility and cost-effectiveness of diagnostic tests and the prevalence of specific *H. pylori* strains within different populations.

Healthcare providers should be trained on these protocols to ensure consistent and accurate application, enhancing the overall effectiveness of *H. pylori* management strategies.

Future Research Directions

Continued research into the development of more efficient, cost-effective, and rapid diagnostic tests is crucial. Such advancements could significantly improve the early detection of resistant *H. pylori* strains, ultimately enhancing treatment outcomes.

Investigate the potential applications of these findings in developing personalized medicine approaches for *H. pylori* treatment, which could include the use of tailored antibiotic therapies based on specific resistance patterns and genetic markers.

By implementing these recommendations, medical practitioners can enhance the precision of *H. pylori* treatments, reduce the incidence of antibiotic resistance, and improve patient outcomes in diverse clinical settings.

Conclusion

The conclusion of the manuscript on *Helicobacter pylori* effectively synthesizes the genetic insights derived from the study, aligning closely with the aims and objectives outlined in the introduction and title. The research has elucidated significant genetic markers associated with the bacterium's resistance to antibiotics and pathogenicity, highlighting the roles of specific genes like *cagA*, *vacA*, and *oipA*. These findings underscore the complexity of *H. pylori* infections and the challenges in treatment due to genetic diversity and antibiotic resistance.

This study's genetic analysis has significant implications for the development of targeted therapies and enhances our understanding of the bacterial mechanisms underpinning resistance and virulence. For instance, the identification of strain-specific genetic markers can lead to more personalized approaches in managing *H. pylori* infections, potentially improving eradication strategies.

However, the study is not without limitations. The genetic findings, while robust, are based on a sample from a specific geographic area, which may not represent the global genetic diversity of *H. pylori*. Future research should focus on expanding the genetic analysis to include strains from varied populations to validate these findings and explore the genetic variability further.

In conclusion, this research adds valuable genetic data to the field of *H. pylori* studies, offering new avenues for understanding the bacterium's resistance mechanisms and pathogenic potential. It is recommended that subsequent studies incorporate broader genetic sampling and explore the interactions between genetic factors and clinical outcomes to fully leverage the potential of genetic insights in combating *H. pylori* infections.

Ethical approval: The study was approved by the ethics committee of Al-Azhar- Assiut Faculty of Medicine Committee No. 68 item 3 dated 12/12/2021 and was conducted by Helsinki standards as revised in 2013. The informed written consent was obtained from all participants.

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