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Helicobacter pylori from Gastric Biopsies: Epidemiology and Burden of Antimicrobial Resistance Against First- and Second-line Drugs

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Abstract:
Objective: The study aimed to detect antibiotic resistance signatures in *H. pylori*.
Methods: Biopsy samples (*n* = 2640) were collected from tertiary care hospital in Peshawar, Pakistan. *H. pylori* was identified using culture, biochemical, and PCR-based methods. The disc diffusion assay was used to determine the antibiotic susceptibility, and multiplex PCR was incorporated for the detection of antibiotic resistance genes.
Results: The PCR detection rate of *H. pylori* among urease-positive isolates (*n* = 489) was 43.9% (*n* = 215). The frequency of *H. pylori* was higher in patients with gastric ulcer (*n* = 53, 33.5%) and gastritis (*n* = 46, 33.8%) samples. In the disc diffusion assay, *H. pylori* exhibited a high resistance rate to first-line drugs (metronidazole, *n* = 214, 99.5%; amoxicillin, *n* = 144, 66.9%; clarithromycin, *n* = 126, 58.6%) compared to second-line drugs (levofloxacin, *n* = 67, 31.1%; tetracycline, *n* = 59, 27.4%). Twenty-four (11.1%) *H. pylori* isolates were resistant to first- and second-line drugs. Molecular detection of *gyrA/gyrB* (*n* = 39, 58.2%), *23S rRNA* (*n* = 69, 54.8%), *frxA/rdxA* (*n* = 91, 42.5%), *16S rRNA* (*n* = 28, 47.4%), and *pbp1* (*n* = 56, 38.8%) genes further confirmed the resistance signatures among *H. pylori* isolates.
Conclusion: The rate of antibiotic resistance was alarmingly high in *H. pylori*. The findings will help in monitoring antibiotic resistance and understanding the associated molecular mechanism for better prevention and control of *H. pylori* infection.
Keywords: Gastric biopsies, *H. pylori*, AMR signatures, first- and second-line drugs

Introduction

Helicobacter pylori is a bacterial pathogen and the causative agent of ulcers, gastritis, and adenocarcinoma.[1] This Gram-negative bacteria exert pathological effects by causing duodenal ulcers and gastritis in 75% of cases, associated with stomach ulcers in nearly 90% of cases, and acting as a risk factor for gastric lymphoma and adenocarcinoma, particularly in Asian, North American, and European populations.[2] The persistent nature of *H. pylori* infections, often beginning in infancy and enduring throughout one's life unless treated with a prolonged course of antibiotics, underscores the complexity and challenges associated with its management.[3] *H. pylori* produces urease, an important virulence factor, facilitating gastric colonization and inducing an inflammatory response.[4]

The World Health Organization reports a staggering 80% prevalence of *H. pylori* infection in developing countries. The prevalence of *H. pylori* infection has increased in certain populations based on genetic predispositions and ethnic factors; however, lower education level, poverty, overcrowding, poor sanitation, and food habits may also have a role.[5] The infection rate is reported to be up to 81% in Pakistan and high as compared to India and Bangladesh.[6] This inconsistency highlights the complicated nature of *H. pylori* distribution, caused by socio-economic aspects, healthcare setup, and antibiotic use.[7]

Antibiotic triple therapy, including clarithromycin, amoxicillin, and metronidazole along with proton pump inhibitors (PPI), has been traditionally used for *H. pylori* eradication.[8] The second-line drugs like levofloxacin, amoxicillin, and metronidazole with PPI have been proven effective to eradicate *H. pylori*.[9] However, the outcome of these treatments is increasingly compromised by the intensifying rates of antibiotic resistance globally.[10] It has been previously reported that *H. pylori* genes, including *rdxA*, *frxA* (metronidazole), *gyrA*, *gyrB* (levofloxacin), *pbp1* (amoxicillin), *16S rRNA* (tetracycline), and *23S rRNA* (clarithromycin), are vulnerable to point mutations that trigger *H. pylori* resistance to antibiotics.[11,12]

The primary factors contributing to the emergence and spread of multidrug-resistant (MDR) *H. pylori* strains globally are associated with the overuse of antibiotics and bacterial-related factors. Antibiotic target alteration, decreased permeability and increased efflux, antibiotic inactivation, and drug extrusion are important mechanisms of MDR.[13,14]

From literature mining, previous studies have primarily reported phenotypic resistance patterns of *Helicobacter pylori*, often based on small sample sizes, limited antibiotic panels, or single diagnostic approaches.[15,16]

The work presents a large-scale, combined phenotypic and molecular analysis of *Helicobacter pylori* antibiotics resistance from gastric biopsy samples. It provides meaningful regional and methodological contributions, particularly relevant to South Asia, where resistance surveillance data remain fragmented. Further, it will advance our current understanding of integrated phenotypic–genotypic resistance analysis and the alarming resistance landscape.

Materials and Methods

Sampling

In this study, patients (*n* = 2640) who had abdominal pain, vomiting, and nausea were included and referred to endoscopy, while patients infected with the hepatitis B virus, hepatitis C virus, and human immunodeficiency virus were excluded from the study. The patient's average age was recorded as 40 years. The study was approved by the Institutional Ethical Review Board of Hayatabad Medical Complex (370/HMC/B&PSC/2020). Informed written consent was taken from all the patients. Tissue samples were cut into small pieces with the help of a sterile blade. The biopsies were transported to the laboratory.

Isolation of H. pylori

All biopsies in thioglycolate broth were inoculated on Columbia blood agar (Oxoid, UK), added with 5% sheep blood and *H. pylori* selective supplement (DENT, Oxoid, Hampshire, UK). The cultures were incubated under microaerophilic conditions using a CampyGen sachet (Thermo Fisher Scientific, Warrington, UK) for 2 to 5 days at 37°C. The Gram staining, oxidase, urease, and catalase tests were performed to identify suspected *H. pylori* as per the previously described protocol.[17] The suspected *H. pylori* isolates were further subjected to DNA extraction.

Genomic DNA Extraction

A DNA kit (Thermo Fisher Scientific, Warrington, UK) was used to extract DNA from suspected *H. pylori* isolates. Briefly, the bacterial pellet was resuspended in proteinase K, followed by incubation at 56°C for three hours. RNase A and lysis solution were added. The column was washed and centrifuged at 10,000 rpm. The column was filled with a dilution buffer and incubated at room temperature. Centrifugation was performed at 14,000 rpm for 5 minutes. Genomic DNA was eluted and measured in a Nanodrop spectrophotometer (Thermo Fisher Scientific, Warrington, UK).

PCR Amplification of Species-specific H. pylori 16S rRNA Gene

The species-specific gene primers, including HP-1(5'-GCGACCTGCTGGAACATTAC-3') and HP-2 (5'-CGTTAGCTGCATTACTGGAGA-3') were used for the identification of *H. pylori*.[18] The PCR reaction mixture (Thermo Scientific, K1081, USA) was prepared by adding 0.4 µl of both primers, 1 µl of DNA, 5 µl of master mix, and 3.2 µl of nuclease-free water. Initial denaturation (95°C) was followed by 35 cycles of denaturation (95°C), primer annealing (58°C), and a final elongation (72°C).

Antibiotic Susceptibility Testing Using Disc Diffusion Assay

Disc diffusion assay was employed to examine the antibiotic susceptibility of the isolate.[19] Mueller-Hinton agar supplemented with 5% sheep blood was utilized. The bacterial solution was adjusted to a McFarland opacity of 0.5. The antibiotic discs (Oxoid, UK), including amoxicillin (AML, 10 µg), tetracycline (TE, 30 µg), levofloxacin (LEV, 5 µg), metronidazole (MTZ, 5 µg), and clarithromycin (CLR, 15 µg), were placed and incubated for 2 to 5 days under microaerophilic conditions with a CampyGen sachet (Oxoid, UK). Zones of inhibition were measured in millimeters according to CLSI 2023 guidelines.[20]

Antibiotic Susceptibility Testing by E-test

E-test (bióMeurieux, France) gradient strip was used as per the manufacturer's protocol.[21] Briefly, *H. pylori* suspension in Columbia broth was inoculated onto a Mueller-Hinton agar plate, and one E-test strip was placed and incubated for 72 hours under microaerophilic conditions with a CampyGen sachet. Minimum inhibitory concentrations (MICs) was noted from the border of the *H. pylori* growth on the E-test.

Multiplex-PCR Detection of Antibiotic Resistance Genes

H. pylori genes contributing to resistance to metronidazole (*rdxA*, 749bp, F: 5-GCCACTCCTTGAACTTTAATTT-3; R: 5-TATGTGCATATCCCCTGTAGG-3), *frxA*, 912bp, F: 5-CGAATTGGATATGGCAGCCG-3; R: 5- TATGTGCATATCCCCTGTAGG-3), levofloxacin (*gyrA*, 347bp, F: 5-TGTCCGAGATGGCCTGAAGC-3; R: 5- TACCGTCATASGTTATCCACG-3, *gyrB*, 345bp, F: 5-CAAACTGGCGGACTGTCAGG-3; R: 5- TTCCGGCATCTGACGATAGA-3), amoxicillin (*pbp1*, 953bp, F: 5-CACGAGCACCGGTAAGATTT-3; R: 5- CGCTATCGTCTGTTCTTTTGG-3), tetracycline (*16S rRNA*, 360bp, F: 5-CAGCGGTAAGATCCTTGAGA-3; R: 5- ACTCGCCGTCGTGTAGATAA-3) and clarithromycin (*23S rRNA* gene, 662bp, F: GATTGGAGGGAAGGCAAT-3; R: 5-CTCCATAAGAGCCAAAGCCC-3) were selected.[22–24] The thermal conditions for multiplex PCR were 95°C for 5 minutes for the initial denaturation, followed by 35 cycles at 95°C for 30 seconds, annealing at 58°C for 20 seconds, extension at 72°C for 30 seconds, and final extension at 72°C for 5 minutes. The amplified product was resolved on a 1.5% agarose gel and visualized in the gel documentation system.

Statistical Analysis

For contingency table 2×2, the Fisher's exact test (https://www.socscistatistics.com) was used. Further, for contingency table 2×3, Fisher's with Freeman–Halton extension (https://www.danielsoper.com) was used. A *P*-value of <0.05 was considered significant. All the experiments were repeated three times. There was no adjustment applied for multiple comparisons, and that subgroup analyses should therefore be interpreted with appropriate caution.

Results

Isolation and Detection of H. pylori

Among the 1478 cultures, 952 (64.4%) were suspected of *H. pylori*. Out of 952, 489 (51.36%) cultures were urease positive. All the urease-positive *H. pylori* suspected cultures were processed for PCR. Among the urease-positive culture, the PCR detection rate was 43.9% ($n = 215$) (Figure 1).

Among urease positive samples ($n = 489$), 194 were obtained from female, while 295 samples were originated from male. On PCR, *H. pylori* detection rate (62.4%, $n = 134$) was high in male as compared to female (37.6%, $n = 81$) (Table 1).

Risk Factors Associated with H. pylori Infection

In risk factors analysis, *H. pylori* detection from the spicy food consumers were significantly high ($P = 0.00001$) on PCR (Table 2).

Gastro-intestinal Pathologies and Frequency of H. pylori Detection

H. pylori detection rate on PCR was higher in gastric cancer ($n = 8$, 42.1%), gastro-duodenitis ($n = 14$, 34.1%) and gastritis ($n = 46$, 33.8%) (Table 3).

Antibiotic Resistance Outline of H. pylori

Antibiogram of five antibiotics including amoxicillin, tetracycline, levofloxacin, metronidazole and clarithromycin was evaluated using disc diffusion assay (Figure 2).

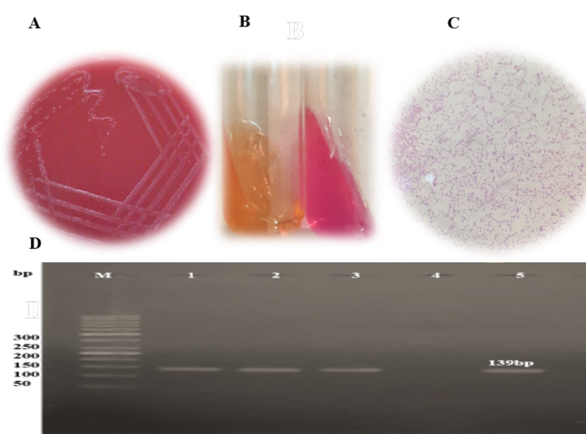

Fig. 1 **Isolation and PCR detection of *H. pylori*: A) Isolates of *H. pylori* on Columbia blood agar medium. B) Urease assay: Yellow slant (urease negative), Pink slant (Urease positive). C) Gram staining of *H. pylori*. D) Molecular identification of *H. pylori* by species-specific primers HP-1 & HP-2; M represents marker (50bp), 1, 2, 3, 5 represent bands of *H. pylori* amplified product (139bp). 4 represents *H. pylori* not detected.**

H. pylori exhibited a high resistance rate (n = 214, 99.5%) against metronidazole. Isolates form gastritis and ulcer patients showed high resistance rate. The Resistance rate to amoxicillin was 66.9%(n = 144). About 126 *H. pylori* isolates (58.6%) were resistant to clarithromycin. The resistance rate to clarithromycin reached to 45.5% (n = 41, P = 0.002) in individuals above 40 years. It was noted that levofloxacin, a second line drug and promising option to eradicate *H. pylori*, encounter increased resistance (n = 67, 31.1%). Levofloxacin susceptibility was significantly higher in gastritis cases. About 27.4% (n = 59) of cases showed resistance against tetracycline; however less susceptibility to tetracycline was noted in ulcer (35.6%) cases (Table 4).

Overall, the 92 (42.7%) *H. pylori* isolate showed multidrug resistance pattern. About 11.1% isolates exhibited resistance to all the five available antibiotics including, amoxicillin, metronidazole, clarithromycin tetracycline and levofloxacin (Table 5).

E-test was performed for all five antibiotics against the resistant *H. pylori* isolates. All the results were in-line with Kirby-Bauer disc diffusion assay (Table 6).

Antibiotic Resistance Genes Among H. pylori Isolates

Among the PCR-positive *H. pylori* isolates, various resistance-associated genes were identified (Figure 3). Specifically, a gene (*pbp1*) associated with amoxicillin resistance was detected in 32 isolates, representing 35.5% of the samples. Further *gyrA/gyrB* (58.2%), *23S rRNA* (54.8%), *16S rRNA* (47.4%)

Table 1. **Overall detection rate and gender demographics of *H. pylori* isolated from gastric biopsies**

Technique	Samples (n)	Not detected n (%)		Detected n (%)		Detection rate (%)
		Female	Male	Female	Male	
Culture	1478	211 (44.5)	264 (55.5)	442 (44.1)	561 (55.9)	67.8
Urease	952	215 (46.5)	248 (53.5)	179 (36.6)	310 (63.4)	51.3
PCR*	489	113 (41.3)	161 (58.7)	81 (37.6)	134 (62.4)	43.9

*Urease positive samples were subjected to PCR.

Table 2. **Risk factors association with *H. pylori* detection on PCR**

Risk factors		Samples	Not detected n (%)	Detected n (%)	P-value
Age group (years)	1–25	48 (9.8)	33 (68.7)	15 (31.3)	0.208
	26–50	231 (47.3)	116 (50.2)	115 (49.8)	
	>50	210 (42.9)	130 (60.9)	85 (40.4)	
Education	Yes	280 (57.3)	164 (59.6)	116 (41.4)	0.330
	No	209 (42.7)	110 (52.6)	99 (47.4)	
Spicy food	Yes	370 (75.7)	201 (54.4)	169 (45.6)	0.00001
	No	119 (24.3)	73 (61.4)	46 (38.6)	
Smoking	Yes	58 (11.9)	34 (58.6)	24 (41.4)	0.349
	No	431 (88.1)	240 (55.6)	191 (44.4)	
Crowded family	Yes	256 (52.4)	154 (60.1)	102 (39.9)	0.686
	No	233 (47.6)	120 (51.5)	113 (48.5)	
Family history	Yes	295 (31.7)	141 (47.8)	154 (52.2)	0.06
	No	194 (60.3)	133 (48.54)	61 (28.4)	

$P < 0.05$ = statistically significant.

Table 3. **Gastro-intestinal findings among urease positive and PCR positive *H. pylori* cases**

Endoscopy findings	Urease positive cases *n* (%)	PCR positive cases *n* (%)
Non ulcer dyspepsia	55 (11.2)	22 (28.5)
Gastric ulcer	105 (21.4)	53 (33.5)
Gastro-esophageal reflex disease	63 (12.8)	25 (28.5)
Gastritis	90 (18.4)	46 (33.8)
Duodenal ulcer	85 (17.3)	33 (27.9)
Gastroduodenitis	27 (5.5)	14 (34.1)
Duodenitis	44 (8.9)	11 (20)
Gastric erosion	9 (1.8)	3 (25)
Gastric cancer	11 (2.2)	8 (42.1)
Total	(*n* = 489)	(*n* = 215)

Fig. 2 **Antibiotic susceptibility of *H. pylori* on Mueller-Hinton agar.**

and *frxA/rdxA* (42.5%) genes further confirmed the resistance pattern (Figure 3, Table 7).

Discussion

H. pylori is the causative agent of gastric pathologies. In developing countries, its prevalence ranges between 85% and 95%, much higher than in developed countries (30% to 50%).[25] In the present study, the PCR detection rate was 43.9% (*n* = 215). The lower detection rate might be due to the selection of urease-positive samples for PCR analysis.

In the current study, the frequency of *H. pylori* resistance to metronidazole was 99.5% (*n* = 214). Metronidazole is a commonly used antibiotic in hospitals for treating dental, genital, and parasitic infections. Our findings are comparable to the resistance rates of 90% and 92.5% reported in Cameroon and Africa.[26] Data on *H. pylori* antibiotic resistance revealed that metronidazole is associated with the highest resistance in South Asia from 1993 to 2009.[27] Detection of *rdxA* and *frxA* (for metronidazole) exhibited a 42.5% (*n* = 91) resistance rate. The lower PCR detection rate suggests that other resistance genes may be involved in metronidazole resistance.

The current investigation found that amoxicillin resistance was present in 66.9% (*n* = 144) of isolates. Similar findings in the Asian population revealed that the frequency of amoxicillin resistance is still low, from 1% to 8.8% and 0% in Taiwan and Japan, respectively.[28] The *pbp1* gene (for amoxicillin resistance) was detected in 38.8% (*n* = 56) of isolates, which might be due to other mechanisms involved in amoxicillin resistance.

The present study revealed a high proportion of clarithromycin resistance in *H. pylori* isolates (*n* = 126, 58.6%). Clarithromycin is the only macrolide commonly prescribed with a proton pump inhibitor, either alone or

Table 4. **Antibiotic resistance pattern and associated demographic factors**

Antibiotics	Gender			Age (years)			Endoscopic findings			
	Male *n* (%)	Female *n* (%)	*P*-value	<40 *n* (%)	>40 *n* (%)	*P*-value	Gastritis	Cancer	Ulcer	*P*-value
Amoxicillin										
Resistant	49 (54.4)	20 (22.2)	0.233	17 (18.8)	52 (57.7)	0.938	28 (31.1)	1 6 (17.7)	225 (27.7)	0.999
Susceptible	12 (13.3)	9 (10)		5 (5.5)	16 (17.7)		7 (7.7)	6 (6.6)	8 (8.8)	
Clarithromycin										
Resistant	35 (38.8)	19 (21.1)	0.015	13 (14.4)	41 (45.5)	0.002	22 (24.4)	8 (8.8)	24 (26.6)	0.519
Susceptible	14 (15.5)	22 (24.4)		20 (22.2)	16 (17.7)		15 (16.6)	0 (0)	21 (23.3)	
Metronidazole										
Resistant	68 (75.5)	21 (23.3)	0.579	18 (20)	71 (78.8)	0.519	30 (33.3)	14 (15.5)	45 (50)	0.963
Susceptible	1 (1.1)	0 (0)		0 (0)	1 (1.1)		1 (1.1)	0 (0)	0 (0)	
Tetracycline										
Resistant	16 (17.7)	12 (13.3)	0.103	12 (13.3)	16 (17.7)	0.264	5 (5.5)	7 (7.7)	16 (17.7)	0.035
Susceptible	24 (26.6)	38 (42.2)		16 (17.7)	46 (51.1)		32 (35.5)	2 (2.2)	28 (31.1)	
Levofloxacin										
Resistant	15 (16.6)	14 (15.5)	0.268	15 (16.6)	14 (15.5)	0.059	20 (22.2)	4 (4.4)	5 (5.5)	0.001
Susceptible	24 (26.6)	37 (41.1)		19 (21.1)	42 (46.6)		16 (17.7)	7 (7.7)	38 (42.2)	

Table 5. **Susceptibility pattern of *H. pylori* to first- and second-line antibiotics**

Antibiotics	**(*n* = 215)**	**%**
Resistance to all antibiotics	24	11.1
Resistance to quadruple	14	6.5
AMX+CLR+MTZ	12	85.7
LEV+MTZ+TE	02	14.2
Resistant to single	98	45.5
MTZ	67	68.3
TE	9	9.1
LVX	11	11.2
AMX	05	5.1
CLR	06	6.1
Resistance to double	56	26
AMX+MTZ	28	50
CLR+ MTZ	18	32.1
LEV+MTZ	10	17.8
Resistance to triple	22	10.2
Resistance to quadruple	14	6.5
AMX+MTZ+CLR+TE	09	64.2
AMX+ CLR+LEV+TE	05	35.7
Sensitive to all antibiotics	1	0.4

AMX (amoxicillin), LEV (levofloxacin), MTZ (metronidazole), CLR (clarithromycin), TE (tetracycline).

Table 6. **Susceptibility profile of resistant *H. pylori* isolates using E-test assay**

Antibiotics	**Clinical breakpoint (mg/l)**	**MIC50 (mg/l)**	**MIC90 (mg/l)**
Metronidazole	>8	7.125	54
Amoxicillin	>0.125	0.011	0.120
Clarithromycin	>0.5	0.022	0.20
Tetracycline	>1	0.034	0.21
Levofloxacin	>1	0.28	8

in combination with a second antibiotic. In the present study, the prevalence of primary clarithromycin resistance is higher than that reported in Turkey (24.8%), Iran (17%), the USA (10% to 15%), Europe (9.9%), Korea (5% to 6%), Hong Kong (4.5%), Germany (2.2%), Malaysia (2.1%), and Canada (less than 4%).[29] The lower detection rate (54.8%, *n* = 69) of the *23S rRNA* gene (for clarithromycin) reflects the existence of other mechanisms associated with clarithromycin resistance.[30]

To cure *H. pylori* infection, tetracycline is usually utilized as part of quadruple treatment. In this study, 27.4% of clinical isolates were resistant to tetracycline. Previous studies reported a 28% tetracycline resistance rate among *H. pylori* isolates. The frequency of tetracycline resistance rate differs considerably across Korea (8.8%), Europe (2.1%), Asia (2.4%), and America (2.7%).[31] Interestingly, the*16S rRNA* gene (associated tetracycline resistance) detected in 47.4% isolates, which might reflect it as marker gene for tetracycline detection.

The fluoroquinolone utilized in the current study was levofloxacin, and the resistance rate was reported as low as 31.1% (*n* = 67), which was also confirmed by *gyrA* and *gyrB* (*n* = 39, 58.2%). Previous studies reported that resistance to fluoroquinolones is uncommon in Africa. Whereas in Europe, a higher prevalence rate (24.1%) than in Asia (11.6%).[32]

Table 7. **Disc diffusion and PCR based resistance pattern of *H. pylori***

Mode of action	**Antibiotics**	**Resistance pattern**		
		Disc diffusion (*n* = 215) *n* (%)	**Gene**	**PCR (*n* = 215) *n* (%)**
Cell wall inhibitor	Amoxicillin	144 (66.9)	*pbp1*	56 (26)
Protein inhibitor	Clarithromycin	126 (58.6)	*23S rRNA*	69 (32.0)
	Tetracycline	59 (27.4)	*16S rRNA*	28 (13.0)
Nucleic acid inhibitor	Metronidazole	214 (99.5)	*frxA*	39 (18.1)
			rdxA	52 (24.1)
	Levofloxacin	67 (31.1)	*gyrA*	21 (9.7)
			gyrB	18 (8.4)

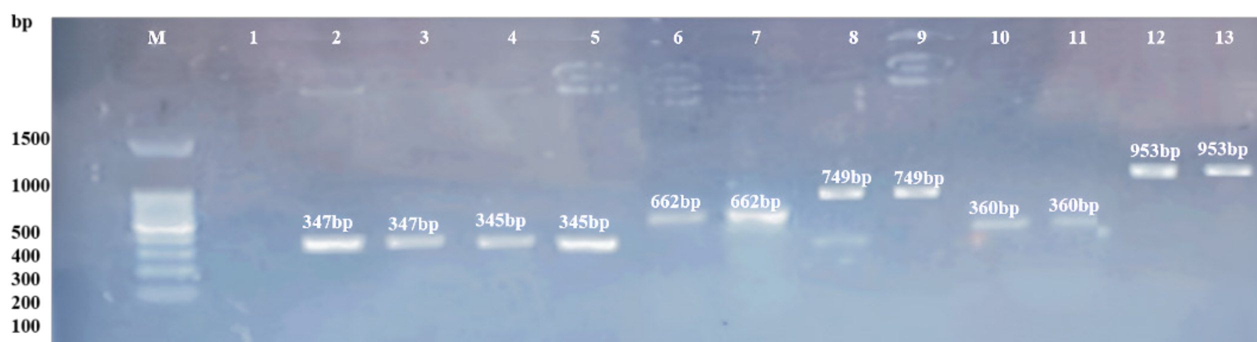

Fig. 3 **Detection of antibiotic resistance genes of *H. pylori*; M = marker (100bp); 1 = negative control; 2, 3 (347bp, *gyraseA*,); 4, 5 (345bp, *gyraseB*); 6, 7 (662bp *23SrRNA*); 8, 9 (749bp, *frxA*); 10, 11 (360bp, *16S rRNA*,); 12, 13 (953bp, *pbp1*).**

Levofloxacin is a DNA synthesis inhibitor and exhibits bactericidal activity. It was previously reported that the eradication rate of *H. pylori* infection was high with levofloxacin as compared to standard triple therapy.[33] This might be due to an increased rate of resistance in *H. pylori* towards clarithromycin and lower prior exposure to levofloxacin.

The findings of the current study indicated that the most prevalent kind of resistance was double drug resistance (26%), followed by triple drug resistance (10.2%). Multidrug resistance is more prevalent in Pakistan than in other countries. *H. pylori* isolates from Mexico were shown to have 30.7% double drug resistance and 8.7% triple drug resistance.[34] The metronidazole resistance was found to be 98.8% in the current study. Such resistance could be a serious threat, reducing the effectiveness of metronidazole-based treatment.[16]

It was noted that there was discordance between phenotypic resistance rates and molecular detection of resistance-associated genes, indicating that the absence or presence of only one gene is not a true marker of resistant or sensitive isolates. There are multiple resistance determinants and resistance mechanisms. For example, the lowest amoxicillin resistance rate via *pbp1* gene detection on PCR reflects that other mechanisms, including *pbp2, pbp3*, β-lactamases (bla_{TEM-1}), efflux pump (*hefA*, *hefC*, *hofH*) and porin (*hopB, hopC*) encoding gene mutations might be responsible for amoxicillin resistance among *H. pylori*.[35–37]

Overall, the resistance rates in the current study are substantially higher than those reported in earlier Pakistani and regional studies, indicating a worsening resistance scenario.[15,16] This study simultaneously evaluated first- and second-line agents with their associated resistance genes, which provided a more comprehensive therapeutic landscape.

It is to note that this study considered the Kirby-Bauer method for initial susceptibility testing due to cost-effectiveness and ease of monitoring antibiotic resistance in Pakistan; however, it is not routinely recommended by CLSI due to lack of breakpoints.

Conclusions

The MDR rate was 42.7% among *H. pylori* isolates. The 11.1% isolates exhibited resistance to all first- and second-line antibiotics. Findings of the study will help to understand the molecular mechanism of antibiotics resistance among MDR *H. pylori* clinical isolates.

Author Contributions

Conceptualization, methodology, project administration, supervision, resources, validation, writing – original draft, writing – review & editing: Muhammad Taj Akbar and Hazir Rahman; investigation, validation: Saneela Shams; validation, writing - review & editing, data curation and formal analysis: Saghir Ahmad; methodology, investigations, resources: Sultan Ayaz; Data curation and formal analysis: All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

The study was conducted in accordance with the Declaration of Helsinki, and approved by IREB of Hayatabad Medical Complex, Peshawar, Pakistan on dated November 20, 2020 (370/HMC/B&PSC/2020).

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

Data Availability Statement

The datasets analyzed during the study are available from the corresponding author upon reasonable request.

Acknowledgments

Not applicable.

Conflicts of Interest

The authors declare no conflicts of interest.

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