Iranian Journal of Microbiology
Volume 18 Number 2 (April 2026) 226-234
DOI: http://doi.org/10.18502/ijm.v18i2.21299

ORIGINAL ARTICLE

Identification of plasmid-mediated quinolone resistance genes in *Helicobacter pylori* isolates from gastrointestinal patients

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Received: February 2025**, Accepted:** December 2025

ABSTRACT

Background and Objectives: The expanding antibiotic resistance of *Helicobacter pylori* isolates is a critical concern for global health. This research aimed to evaluate the prevalence of PMQR genes, including *qnrA1-6*, *qnrB4*, *qnrB1*, *qnrS*, *qepA*, and *aac(6´)Ib-cr*, among *H. pylori* strains isolated from patients at Qazvin Hospital.

Materials and Methods: In this study, 212 patients with gastrointestinal diseases who were referred to Qazvin Hospital underwent endoscopy, and gastric biopsies were collected. *H. pylori* isolates were confirmed by biochemical and molecular tests. PCR was performed to detect the virulence genes *babA2* and *sabA*. Subsequently, the presence of the PMQR genes *qnrA1-6*, *qnrB4*, *qnrS*, *qnrB1*, *qepA*, and *aac(6')Ib-cr* were molecularly identified.

Results: 149 (70%) of isolates were phenotypically and molecularly confirmed as *H. pylori* isolates. Of the 149 isolates, 102 isolates (68.45%) with *babA2* and 120 isolates (80.53%) with *sabA* genes were identified. frequencies of the genes *qnrA1-6*, *qnrB4*, *qnrB1*, and *qepA* were 8 (5.36%), 4 (2.68%), 12 (8.05%), and 13 (8.72%), respectively. Notably, *qnrS* and *aac(6´) Ib-cr* genes were not detected in isolates.

Conclusion: The findings of this study confirm that regular monitoring of antibiotic resistance genes is essential to prevent the further spread of PMQR resistance genes and to optimize clinical decision-making.

Keywords: *Helicobacter pylori*; Quinolones; Drug resistance; Plasmid; Gastrointestinal diseases

INTRODUCTION

Helicobacter pylori predominantly colonizes the gastric antrum in humans, facilitated by its flagella, which enable bacterial motility (1). Statistical data indicate that the annual incidence of *H. pylori* infection ranges from approximately 4-15% in developing countries to about 0.5% in developed nations, highlighting its significant global prevalence. Interestingly, 10-20% of individuals remain uninfected by *H. pylori*, suggesting potential factors that confer natural resistance or reduced susceptibility (2, 3).

The virulence factors of *H. pylori* play a vital role in facilitating its colonization, survival, and pathogenicity within the gastric environment. The urease enzyme neutralizes stomach acidity by producing ammonia, enabling the bacterium to survive in harsh acidic conditions. The CagA protein, which is encoded by the *cag* pathogenicity island, disrupts cellular signaling pathways and induces inflammation, sig-

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nificantly increasing the risk of gastric cancer. VacA toxin contributes by forming vacuoles in epithelial cells, impairing mitochondrial function, and suppressing immune responses. Additionally, flagella are crucial for motility and successful colonization of the gastric mucosa, while adhesions, such as BabA and SabA, facilitate attachment to gastric epithelial cells. Collectively, these factors contribute to the onset and progression of gastritis, peptic ulcers, and gastric cancer (4, 5).

The global importance of studying and addressing this infection stems from its association with several prevalent gastric and esophageal disorders, such as gastritis, duodenal ulcers, peptic ulcers, gastric adenocarcinoma, and gastric lymphoma (6). Therefore, the treatment of *H. pylori* infection is of significant importance. The standard approach to managing this significant gastrointestinal pathogen involves the use of antibiotics such as metronidazole, amoxicillin, clarithromycin, tetracycline, furazolidone, ciprofloxacin, and rifamycin. These are typically administered in combination with proton pump inhibitors and bismuth salts (7, 8).

However, treating *H. pylori* infections is becoming more difficult because of the increasing resistance of bacterial strains to antibiotics. Consequently, clinicians are compelled to adopt multidrug regimens to achieve successful eradication (8). In certain countries, the standard triple therapy continues to be the first-line treatment for *H. pylori* infection. According to the Maastricht consensus report, this regimen is effective in regions where *H. pylori* resistance to clarithromycin is less than 15% (9).

Unfortunately, studies conducted in Iran indicate a rising trend of antibiotic resistance in *H. pylori* (10). Resistance to quinolones has been reported at a rate of 21.9% (11). The primary mechanisms underlying resistance to quinolone antibiotics include mutations in drug targets and reduced antibiotic permeability (12, 13).

Levofloxacin is a fluoroquinolone antibiotic widely used to treat various bacterial infections, including respiratory, urinary, and skin infections, as well as *Helicobacter pylori*-associated diseases. It exerts its antimicrobial effect by targeting essential bacterial enzymes, DNA gyrase (*gyrA*) and topoisomerase IV (*parC*), inhibiting DNA replication and ultimately leading to bacterial cell death. The *qnr*, *qepA*, and *aac(6´)Ib-cr* genes play a crucial role in antibiotic resistance, particularly against fluoroquinolones. These genes are plasmid-mediated and can spread among bacterial species, facilitating the dissemination of resistance. The *qnr* genes, including *qnrA*, *qnrB*, and *qnrS*, encode proteins that protect DNA gyrase and topoisomerase IV, the primary targets of fluoroquinolones, from their inhibitory effects. The *qepA* gene encodes an efflux pump that expels fluoroquinolones from the bacterial cell, reducing the intracellular concentration of the drug and its efficacy. Similarly, the *aac(6´)Ib-cr* gene modifies fluoroquinolones through acetylation, diminishing their activity. Clinically, these genes are significant as they contribute to treatment failures, particularly in infections like *H. pylori*, where effective combination therapies are essential. The existence of these resistance genes reduces the efficacy of standard treatments, increases the prevalence of multidrug-resistant infections, and limits therapeutic options, underscoring the importance of their detection for developing diagnostic tools and novel treatment strategies. Additionally, mechanisms like reduced drug permeability and overexpression of efflux pumps contribute to resistance but are less significant compared to genetic mutations. Studies focusing on *gyrA* and *parC* mutations aim to identify resistance patterns, optimize treatment regimens, and minimize therapeutic failures (14, 15).

In Iran, resistance to levofloxacin varies based on geographic region and infection type. For example, a study in Bushehr reported a resistance rate of approximately 6% in blood and urine isolates (16), while other studies identified higher resistance rates among Enterobacteriaceae. These findings highlight regional differences and the importance of localized surveillance (17). In contrast, recent studies have highlighted levofloxacin-containing regimens as a promising alternative for first-line eradication therapy of *H. pylori*. Levofloxacin demonstrates a broad spectrum of activity against a wide range of bacterial pathogens (18, 19).

Nonetheless, there have been instances of resistance to this antibiotic. Given the high resistance rates of *H. pylori* to commonly used antibiotics and the global prevalence of this infection—characterized by significant geographic variations—the rapid increase in antibiotic resistance in this bacterium poses a serious challenge. Therefore, addressing *H. pylori* infections requires urgent consideration of evolving resistance patterns and the development of alternative treatment strategies (20).

Variability in the determination of antibiotic susceptibility in *H. pylori* has been observed across different studies. This discrepancy may stem from several factors, including the inappropriate selection of antibiotics, excessive and indiscriminate antibiotic use, inaccuracies in determining the required duration of therapy, the inability of certain laboratories to culture *H. pylori* effectively, and subsequently perform antibiotic susceptibility testing, as well as the failure to adhere to standard protocols during various stages of susceptibility testing (21-23).

The effective application of microbiological diagnostics for accurate detection and prevention of resistant pathogens can significantly reduce the need for antimicrobial use. Proper infection control measures greatly benefit patients by lowering mortality rates globally. Expertise in microbiology laboratories is critical for identifying resistant bacteria. Misdiagnosis or errors in laboratory testing may lead to bacteria being incorrectly reported as resistant or susceptible, resulting in inappropriate drug administration. This, in turn, facilitates the transmission of bacteria to other patients (24).

Consequently, identifying bacteria with concealed resistance mechanisms is essential. Reliable methods for detecting resistance mechanisms are necessary. Hence, the objective of the current study is to examine the plasmid-mediated resistance patterns to levofloxacin in *H. pylori* isolates obtained from patients visiting Velayat Hospital in Qazvin, Iran.

MATERIALS AND METHODS

Study population and sampling method. This cross-sectional study was conducted on *H. pylori* isolates obtained from biopsy samples submitted to the laboratory of the educational Velayat Hospital in Qazvin-Iran from November 2022 to January 2024. The study utilized a descriptive approach, and sample size estimation was performed using the formula for proportion estimation. Considering a 95% confidence interval, an anticipated resistance prevalence of 55% (25), and a margin of error of 8% (0.08), the sample size was calculated to be 149.

Inclusion and exclusion criteria. The inclusion criteria comprised biopsy samples that tested positive for the presence of *H. pylori*. The exclusion criteria were as follows: (1) Individuals under 18 years of age, (2) Patients with a history of gastric surgery, (3) Patients who had taken antibiotics within the past month or medications such as bismuth within two weeks prior to sampling, (4) Individuals with occult blood tests positive or coagulation disorders, and (5) Duplicate biopsy samples submitted for the same patient.

Culture of *H. pylori*. Biopsy samples were collected from patients with gastrointestinal disorders following their informed written consent and completion of a patient information questionnaire. During endoscopy, two biopsy samples were obtained (the gastric antrum and the corpus regions) from each patient. One sample was placed in a semi-solid thioglycolate transport medium at 4°C until its transfer to a solid Brucella agar culture medium. The Brucella agar (Merck) was supplemented with 10% fetal bovine serum (Gibco), defibrinated sheep blood and *H. pylori* selective supplement (Ibresco) (Vancomycin 0.01 g, Amphotericin B 0.005 g, Cefsulodin 0.01 g and Trimethoprim 0.02 g). The second sample was used for a rapid urease test by placing it on a rapid urease slide. Finally, both samples were sent to the Qazvin Medical Microbiology Research Center for further studies. For the first sample, after inoculation onto the culture medium, the plates were incubated under microaerophilic conditions with 5-10% CO_2 at 37°C for 6-8 days. Grown colonies were subjected to confirmatory tests, including morphological evaluation of the colony type, gram staining, and biochemical assays such as catalase, oxidase, and urease positivity, to confirm the presence of *H. pylori* (26).

DNA extraction. DNA extraction from the grown *H. pylori* isolates was performed using the QIAamp DNA Mini Kit (Qiagen) following the manufacturer's instructions. The extracted DNA samples were stored at -20°C for further analysis. The quality of the extracted DNA was assessed using a Thermo Scientific ™ NanoDrop™ Micro volume spectrophotometry in 260/280 nm.

Detection of virulence genes in clinical *H. pylori* isolates. *16S rRNA* gene was used for molecular confirmation of *H. pylori* isolates. PCR was used to identify virulence genes *babA2* and *sabA* in *H. pylori* isolates. The PCR reactions were conducted using primers listed in Table 1 and temperature profile in Table 2. The PCR products were then analyzed on

Table 1. Primers used for the detection of *babA2*, *sabA* and *16S rRNA* genes

Gene	Sequences	Annealing°C	Product (bp)	References
babA	F-CCAAACGAAACAAAAAGCGT	59	105	This study
	R- GCTTGTGTAAAAGCCGTCGT			
sabA	F-CTCTCTCTCGCTTGCGGTAT	59	187	This study
	R-TTGAATGCTTTGCCTCAATG			
16S rRNA	F- GCGCAATCAGCGTCAGGTAATG	53	522	(27)
	R- GCTAAGAGAGCAGCCTATGTCC			

Table 2. The temperature profile for PCR

PCR steps	Heat and Time
Primary denaturation	95°C for 5 min
Denaturation	95°C for 5 min
Extension	72°C for 1 min
Go to repeat	30 cycles
Final extension	72°C for 10 min

1.2% agarose gel electrophoresis to confirm the presence or absence of the target genes. The primers used in this study were designed using Beacon Designer 7 software.

Evaluation of resistance gene frequency. The presence of *qnrA1-6*, *qnrB4*, *qnrS*, *qnrB1*, *qepA*, and *aac(6´)Ib-cr*—plasmid-mediated quinolone resistance genes—was assessed in *H. pylori* isolates. PCR was performed using the primers listed in Table 3 and temperature profile in Table 4. The primers used in this study were designed based on previously published methodology. PCR products were analyzed by 1.2% agarose gel electrophoresis to determine the presence or absence of the target resistance genes. To ensure the validity of the tests, confirmed strains were used as positive controls, while reactions with no template DNA served as negative controls. Additionally, microtubes containing reaction components without DNA template were included as procedural controls to verify the reliability of the assay. The primers used in this study were designed using Beacon Designer 7 software.

Data analysis. Data analysis was performed using the chi-square test and Fisher's exact test. Statistical computations were carried out using SPSS software, version 19. A p-value less than 0.05 was considered statistically significant.

RESULTS

***H. pylori* isolates.** In this cross-sectional study, out of 212 patients with gastrointestinal diseases, 149 were included. All these patients tested positive for the rapid urease test (RUT), indicating infection with *H. pylori.* The clinical characteristics of the patients are summarized in Table 5.

Demographic results of the patients. The demographic and clinical characteristics of the 149 patients included in the study revealed an age range of 19-80 years, with a mean age of 46.20 ± 13.16 years. Among the participants, 59.73% were male, and 40.27% were female. Endoscopic evaluations showed that 51% of patients had peptic ulcer disease (PUD), while 8% presented with intestinal metaplasia, 18% with duodenal ulcers, 18% with chronic gastritis (CG) and 5% with hiatus hernia. Additional assessments indicated that 23.48% of patients were smokers. These findings underscore the diverse clinical and demographic profiles of the studied population.

Results of *H. pylori* culture and confirmation. After 5 days of incubation, 149 positive cultures were observed on brucella blood agar medium. Gram staining of the colonies revealed spiral-shaped bacteria, confirming their gram-negative morphology (Fig. 1). Catalase, oxidase, and urease tests were performed to further validate the bacterial isolates (Table 6). These findings confirm the successful isolation and identification of *H. pylori* in the study population.

Result of detection of virulence genes in clinical *H. pylori* isolates. Out of the 149 isolates identified through phenotypic screening, the molecular PCR analysis revealed the presence of specific genes as follows: 102 isolates (68.45%) for *babA2* and 120 isolates (80.53%) tested positive for *sabA* using specific

Table 3. Primers used for the detection of *qnrA1-6*, *qnrB4*, *qnrS*, *qnrB1*, *qepA*, and *aac(6´)Ib-cr* genes

Gene	Sequences	Annealing °C	Product (bp)	References
qnrS	F-CCT ACA ATC ATA CAT ATC GGC R-GCT TCG AGA ATC AGT TCT TGC	55	621	This study
qnrB1	F-GGC ACT GAA TTT ATC GGC R-TCC GAA TTG GTC AGA TCG	50	430	This study
qnrB4	F-AGT TGT GAT CTC TCC ATG GC R-CGG ATA TCT AAA TCG CCC AG	54	358	This study
qnrA1-6	F-ACG CCA GGA TTT GAG TGA C R-CCA GGC ACA GAT CTT GAC	53	564	This study
qepA	F-GCA GGT CCA GCA GCG GGT AG R-CTT CCT GCC CGA GTA TCG TG	58	218	This study
aac(6´)Ib-cr	F-TTG CGA TGC TCT ATG AGT GGC TA R-CTC GAA TGC CTG GCG TGT TT	58	482	This study

Table 4. The temperature profile for PCR

PCR steps		Heat and Time
Primary denaturation		95°C for 5 min
Denaturation		95°C for 5 min
	genes	
Annealing	*qepA* and *aac(6´)Ib-cr*	58°C for 45 min
	qnrS	55°C for 45 min
	qnrB1	50°C for 45 min
	qnrB4	54°C for 45 min
	qnrA1-6	53°C for 45 min
Extension Go to repeat Final extension		72°C for 1 min 30 cycles 72°C for 10 min

Table 5. Clinical characteristic of 149 *H. pylori* positive patients

Clinical status	% (n=149)
Rapid urease test	100% (149/149)
Abdominal pain	87.24% (130/149)
Smoker	23.48% (35/149)
Hypertensive gastropathy and anemia	2.68% (4/149)
Antibiotic use in the last three months	26.17% (39/149)

primers. These findings further confirmed the identity of *H. pylori* and highlighted the distribution of virulence-associated genes within the isolates.

Molecular test results for the frequency of *qnrA1-6, qnrB4, qnrS, qnrB1, qepA,* and *aac(6´) Ib-cr* genes. PCR testing was performed on 149 *H. pylori* isolates to determine the frequency of the resistance genes *qnrA1-6*, *qnrB4*, *qnrS*, *qnrB1*, *qepA*, and *aac(6´)Ib-cr.* As shown in Table 7, *qnrB1* and *qepA* were the most prevalent genes among the isolates tested. Table 8 displays the co-occurrence patterns of these resistance genes. According to the results, the most frequent co-occurrence was observed between *qnrB1* and *qepA*, which was identified in 2.68% of the isolates.

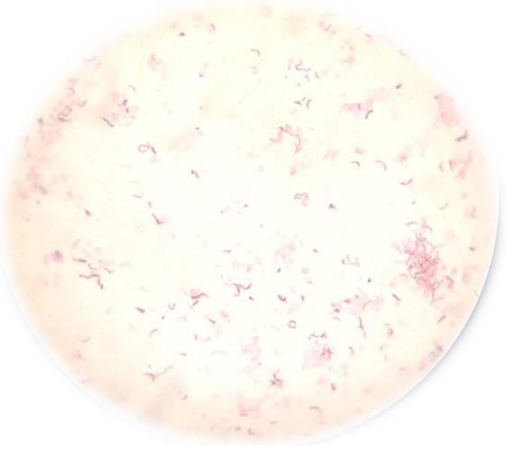

Fig. 1. Gram stain image of *H. pylori* bacteria

DISCUSSION

This study examines the demographic and clinical characteristics of 149 patients, revealing significant trends in gastrointestinal disorders. The average age of the participants was 46.20 ± 13.16 years, with male individuals comprising the majority (59.73%)

Table 6. Phenotypic test results for identification of *H. pylori* isolates

Colonies growth morphology on agar plate	Urease test	Catalase test	Oxidase test	Gram staining
H. pylori colony	149 (70.28%)	149 (70.28%)	149 (70.28%)	149 (70.28%)
Non-*H. pylori* colony	63 (29.71%)	63 (29.71%)	63 (29.71%)	63 (29.71%)
Total number of biopsies performed in this study	212	212	212	212

Table 7. Frequency of genes *qnrA1-6*, *qnrB4*, *qnrS*, *qnrB1*, *qepA*, and *aac(6´)Ib-cr* in 149 isolates

Genes	Frequency (%)
qnrS	0 (0)
qnrB1	12 (8.05)
qnrB4	4 (2.68)
qnrA1-6	8 (5.36)
qepA	13 (8.72)
aac(6´)Ib-cr	0 (0)
Total Samples	149 (100)

Table 8. Comparison of simultaneous results of resistance genes in 149 isolates

Genes	(Frequency %)
qnB1 + qnrB4	1 (0.67)
qnrB1 + qnrA1-6	1 (0.67)
qepA + qnrA1-6	1 (0.67)
qnrB4 + qepA	1 (0.67)
qnB1 + qepA	4 (2.68)
Total Samples	8 (5.36)

of the cohort. These findings are consistent with studies indicating a higher prevalence of gastrointestinal disorders among middle-aged individuals and males, reflecting both lifestyle factors and hormonal influences. The study also highlights lifestyle factors: 23.48% of patients were smokers. Smoking prevalence aligns with findings from Ohlsson et al. (2017), where 20-25% of patients with gastrointestinal disorders reported smoking (28). The findings of this study are consistent with existing literature regarding the demographic and clinical characteristics of patients with gastrointestinal disorders, with slight variations likely reflecting regional, dietary, or cultural influences. Further research could explore these factors in greater depth to clarify their impact on disease prevalence and progression.

Analysis of the genotypic profiles showed a high prevalence of the pathogenicity genes *babA* and *sabA* in the *H. pylori* isolates. This high prevalence is consistent with previous reports by Zhao et al. (2020) and Sheu et al. (2010), who also found these virulence genes to be commonly associated with *H. pylori* infections (29, 30). The presence of such virulence factors is known to contribute to gastric inflammation, tissue damage, and ultimately disease development.

The rising resistance of *H. pylori* to fluoroquinolones poses a major obstacle to the successful treatment of infections caused by this pathogen. This challenge is further exacerbated by the emergence of plasmid-mediated resistance mechanisms. Notably, resistance genes such as *qnrA1-6*, *qnrB1*, *qnrB4*, *qnrS*, *qepA*, and *aac(6´)Ib-cr* play a crucial role in reducing bacterial susceptibility to fluoroquinolones, complicating therapeutic strategies. Levofloxacin resistance in *H. pylori* is a growing concern globally. It underscores the need for careful antibiotic use, improved diagnostic methods, and the development of alternative treatments. Proactive management strategies, including susceptibility testing and antibiotic stewardship, are essential to preserving the efficacy of levofloxacin and improving treatment outcomes.

In the present study, 149 clinical *H. pylori* isolates were evaluated for the presence of plasmid-mediated quinolone resistance genes. The distribution of these genes was as follows: *qnrS*, 0 (0%); *qnrB1*, 12 (8.05%); *qnrB4*, 4 (2.68%); *qnrA1-6*, 8 (5.36%); *qepA*, 13 (8.72%); and *aac(6')Ib-cr*, 0 (0%). Notably, *qnrS* and *aac(6')Ib-cr* were not detected in any of the isolates.

In our molecular investigation, we found varying frequencies of resistance-associated genes, with *qepA* (8.72%) and *qnrB1* (8.05%) being the most prevalent. These results align with global data reporting the emergence of *H. pylori* strains harboring plasmid-mediated quinolone resistance (PMQR) genes. The absence of *qnrS* and *aac(6')-Ib-cr* genes in our isolates mirrors findings from Mégraud et al. (2021), who reported variable prevalence rates for these genes across European regions (31). In a study

conducted in China, the frequency of mutations related to levofloxacin resistance was reported to be over 50%, indicating the widespread use of fluoroquinolones in this region (32). In Europe, the average resistance rate is reported to be around 15-30%. In Iran, several studies have reported a variable resistance rate ranging from 25% to 40%. In a study by Rezaei et al. (2024) in Tehran, specific mutations in codons 87 and 91 of the *gyrA* gene were identified, reflecting a high rate of resistance (33).

In a study by Abadi et al., 150 out of 170 patients (88.2%) tested positive for *H. pylori*, and 150 individual colonies were collected for levofloxacin resistance testing, which showed a 5.3% resistance rate. This was the first study on *H. pylori* resistance to levofloxacin in Iran (34). In another study by Mokhtari et al. (35), 19.66% of *H. pylori* strains were sensitive to all antibiotics tested, with nalidixic acid showing the highest resistance (52.14%), and levofloxacin the lowest resistance (37.61%). Among the remaining strains, 34.8% were resistant to at least one quinolone, with *qnrA*, *qnrB*, and *qnrS* detected in 15.19%, 30.88%, and 72.78%, respectively.

These resistance rates are higher than those observed in the present study. Similarly, Bluemel et al. (36) reported a 13.4% levofloxacin resistance rate in *H. pylori* isolates, which also exceeds our findings. Additionally, Yan et al. (37) documented higher prevalence rates of the qnr and *aac(6')-Ib-cr* genes (8.5% and 10.8%, respectively) compared to our study. Overall, studies in Iran and other countries indicate that the emergence of quinolone-resistant bacterial isolates is increasing, leading to higher drug resistance and treatment failure, which poses a significant challenge to healthcare systems.

While the role of these genes is well-established in Enterobacteriaceae, limited studies have reported their presence in *H. pylori*. Given the bacterium's genomic plasticity and its ability to acquire resistance through horizontal gene transfer, the potential presence of these genes in *H. pylori* isolates necessitates thorough investigation. The identification of these plasmid-mediated resistance determinants in *H. pylori* would significantly expand our understanding of its resistance mechanisms and highlight the importance of plasmid-mediated gene transfer in its adaptation. Resistance rates to fluoroquinolones vary by region, with higher rates reported in areas with frequent antibiotic misuse. Studies in Iran indicate increasing resistance to fluoroquinolones, but data on the specific contribution of genes like *qnrA1-6*, *qnrB4*, *qnrS*, *qnrB1*, *qepA*, and *aac(6´)Ib-cr* are scarce. Expanding surveillance to detect these genes can provide valuable insights into resistance dynamics and inform tailored treatment strategies.

CONCLUSION

The identification of plasmid-mediated resistance genes in *H. pylori* may represent a novel mechanism driving the increasing resistance rates. This study highlights the critical need for molecular surveillance to detect emerging resistance determinants and guide effective therapeutic strategies. Future research should focus on the co-occurrence of these genes and their impact on fluoroquinolone efficacy in *H. pylori*. Additionally, strategies to limit antibiotic misuse and promote alternative therapeutic approaches are imperative. Monitoring the prevalence of resistance genes, such as *qnrA1-6*, *qnrB1*, *qnrB4*, *qnrS*, *qepA*, and *aac(6´)Ib-cr*, is crucial in addressing the global challenge of antibiotic resistance. Routine evaluation of resistance genes prevalence is an indispensable tool in combating antibiotic resistance. It provides actionable insights for clinicians, researchers, and policymakers, enabling the development of strategies that are both regionally relevant and globally impactful. This proactive approach ensures that resistance mechanisms are identified and addressed before they become widespread.

ACKNOWLEDGEMENTS

The authors would like to thank the Medical Microbiology Research Center, Qazvin, University of Medical Sciences for financial support (grant project number 784). This study is approved under the ethical approval code of the Ethics Committee of Qazvin University of Medical Sciences, Qazvin, Iran (IR.QUMS.REC.1401.204).

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