Genetic basis for metronidazole and clarithromycin resistance in *Helicobacter pylori* strains isolated from patients with gastroduodenal disorders

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Background: The aim of this study was to evaluate the antimicrobial resistance and genetic basis for metronidazole (Mtz) and clarithromycin (Cla) resistance in strains of *Helicobacter pylori*, isolated from patients with gastroduodenal disorders.

Patients and methods: A total of 157 *H. pylori* isolates (from 22 gastric cancer, 38 peptic ulcer disease, and 97 non-ulcer dyspepsia patients) were analyzed for drug susceptibility to Mtz and Cla, by gradient diffusion test (E-test, MAST). The PCR and sequence analysis of the *rdxA* and *frxA* for Mtz-resistant strains and the 23S rRNA for Cla-resistant strains were used to determine the genetic basis of drug resistance in *H. pylori* strains. Increased expression of TolC homologous genes (*hefA*) that upregulates efflux pump activity was determined in multidrug-resistant (MDR) strain of *H. pylori* by real-time PCR technique.

Results: Among 157 *H. pylori* isolates, 32 (20.4%) strains were resistant to at least one of the antimicrobial agents. The highest resistance rate was attributed to Mtz (n=69, 43.94%). Among the resistant strains of *H. pylori*, 15 cases (9.55%) were detected as MDR. Mutations in the *rdxA* (85.5%) and A2143G point mutations (63.1%) in the 23S rRNA were the most common cause of resistance to Mtz and Cla in strains of *H. pylori*, respectively. In MDR strains, the *rdxA* mutation and A2143G-point mutation in the 23S rRNA were the most abundant mutations responsible for drug resistance. The relative expression of *hefA* in MDR strains (mean 3.706) was higher than the susceptible strains (mean 1.07).

Conclusion: Mutational inactivation and efflux pump overexpression are two mechanisms that increase the resistance to *H. pylori* antimicrobial agents and the rate of MDR strains. In Iran, the mutations of *rdxA* and *frxA* in Mtz-resistant strains and A2143G and A2142G of the 23S rRNA in Cla-resistant strains were significant. The screening for these mutations could help to prevent antibiotic resistance, and to determine the most effective anti-*H. pylori* drugs.

Keywords: *H. pylori*, drug resistance, efflux pump, genetic mutations

Introduction

*Helicobacter pylori* is an etiological factor for gastroduodenal disorders such as chronic gastritis, peptic ulcer disease (PUD), and in more severe cases, *H. pylori* infection may result in gastric cancer (GC).1 Treatment of *H. pylori* infection is usually a challenge because the strains of *H. pylori* are susceptible to most antimicrobial agents in vitro, but in vivo the successful treatment is hard.2 Treatment process is usually performed by a combined therapy regimen that recommended eradicating the *H. pylori* infection. The emergence of antibiotic resistance is considered as the major cause of treatment failure.3,4 A successful combination therapy of a proton pump inhibitor and simultaneous prescription of two or three kinds of antibiotics, including tetracycline (Tet),
metronidazole (Mtz), clarithromycin (Cla), and amoxicillin (Amx) are recommended for treatment of *H. pylori* infection. The prevalence of antibiotic resistance of strains of *H. pylori* varies in different geographical regions, and is associated with the consumption of antibiotics in those areas. In recent years, the widespread use of antibiotics has increased the rate of resistance of *H. pylori* to standard therapies. Resistance to Mtz and Cla has increased worldwide, and also multidrug-resistant (MDR) strains of *H. pylori* and simultaneous resistant to Mtz, Cla, and Amx have been reported. Recent studies suggest that the underlying antibiotic resistance mechanisms are mainly due to the genetic plasticity of *H. pylori* that results in genetic mutations. These include point mutation, involving substitution of A to G at positions 2142 and 2143, in the domain V of the 23S rRNA, which confers resistance toward Cla. Similarly, mutations in the *rdxA* and *frxA* are reported to confer Mtz resistance in *H. pylori*. Mutational patterns in the 23S rRNA, *rdxA*, and *frxA* are caused by the inactivation of the gene function via frameshift mutation, insertions, and deletions, and showed wide geographical divergence.

In addition, possible mechanisms of intrinsic drug resistance involve decreased drug uptake or increased drug efflux. Efflux of compounds is a phenomenon commonly observed in bacteria. Five families of multidrug efflux transporters have been described. One of them, widespread in Gram-negative bacteria, is the resistance-nodulation-division (RND) family of efflux systems. There are three RND families: *hefABC*, *hefDEF*, and *hefGHI*. The *hefA*, *hefD*, and *hefG* are the *TolC* homolog, encoding the outer membrane protein belonging to efflux pump. About 69% of the Iranian population were infected by *H. pylori*, and failure in treatment involve decreased drug uptake or increased drug resistance due to the genetic plasticity of *H. pylori* that result in genetic mutations. These include point mutation, involving substitution of A to G at positions 2142 and 2143, in the domain V of the 23S rRNA, which confers resistance toward Cla. Similarly, mutations in the *rdxA* and *frxA* are reported to confer Mtz resistance in *H. pylori*. Unlike mutational patterns in the 23S rRNA, the mutations in the *rdxA* and *frxA* are caused by the inactivation of the gene function via frameshift mutation, insertions, and deletions.

**Patients and methods**

**Ethics statement**

The study was approved by the Research Ethics Committee of the Ahvaz Jundishapur University of Medical Sciences (No: IR.AJUMS.REC.1395.220.), Ahvaz, Iran. Written informed consent was obtained from all the gastroduodenal patients.

**Bacterial culture and DNA isolation**

A total 157 *H. pylori* (22 GC, 38 PUD, and 97 non-ulcer dyspepsia [NUD]) strains originally isolated between 2015 and 2016 from 150 patients living in Ahvaz, Khuzestan province, Iran, were selected. The patients who had not received nonsteroidal anti-inflammatory drugs or any anti-*Helicobacter* therapy at least for 3 months prior to endoscopy were included. Upper gastro-endoscopy was performed on patients’ with clinical symptoms, including gastritis, abdominal pain, weight loss, epigastric pain, vomiting, dyspepsia and history of GC, melena, anemia, and history of gastric ulcer for biopsy collection. Brucella agar (EMD Millipore, Billerica, MA, USA), supplemented with 7% defibrinated sheep blood, 7% fetal calf serum (FCS), 10 mg/L vancomycin, 5 mg/L amphotericin B, 5 mg/L trimethoprim, and 20 U/mL polymyxin B, was used to culture the *H. pylori* strains under microaerophilic conditions (10% CO₂, 5% O₂, and 85% N₂; EMD Millipore) for 5–7 days, and all the plates were incubated at 37°C with 100% humidity. Based on colony morphology (small and translucent colonies), bacterial shape (curved), and a Gram-negative stain, and also using biochemical tests, including urease, catalase, and oxidase, preliminary detection of *H. pylori* was performed. After sub-culturing a colony (twice or thrice) on the Brucella agar, supplemented with 7% sheep blood, all the isolates were stored in skim milk with 20% glycerol and 7% FCS at −70°C. Genomic DNA was extracted from antral biopsy and the colonies using the high pure DNA extraction kit (Hoffman-La Roche Ltd., Basel, Switzerland), according to the manufacturer’s instruction. Then, the extracted DNA was quantified, using a biophotometer through measurement of the associated OD at 260 nm (Eppendorf, Hamburg, Germany).

**Detection of *H. pylori* by PCR assay**

For preliminary detection of *H. pylori* strains, the PCR assay was performed using the primers based on the *H. pylori* 16S rDNA. PCR assays were performed using one set of oligonucleotides (Hpx1/Hpx2) (5′-CTGGAGARAC-
TAAGYCTTCC-3′ and 5′-GAG-GATAACTCATGCG-GAAGCGGA-3′), amplifying a 150-bp fragment.\(^9\) PCR assay was carried out in a thermocycler nexus gradient (Eppendorf). A 25 \(\mu\)L final reaction, including 10\(\times\) PCR buffer, 1.5 mM MgCl\(_2\), 10 mM dNTPs, 0.5 \(\mu\)M of each primer, 1.5 U super Taq polymerase, and 5 \(\mu\)L of template DNA was prepared for PCR assay. The cycling parameters were 40 cycles of 95°C for 5 minutes, 56°C for 1 minute, and 72°C for 1 minute. The amplified products were detected by electrophoresis on a 2% agarose gel containing ethidium bromide (500 ng/mL). The bands were visualized using A10 system (Bio-Rad Laboratories Inc., Hercules, CA, USA).

**Antibiotic susceptibility testing**

Bacterial colonies obtained during 72-hour culture were suspended in the brain heart infusion broth with a density of three on the McFarland scale, 10\(^6\) cells CFU/mL. In the next stages, the bacterial suspension was inoculated on Mueller-Hinton medium (Becton Dickinson, Franklin Lakes, NJ, USA), supplemented with 10% defibrinated sheep blood. Then E-test antibiotic impregnated with a gradient of concentration (MIC) for the growth of bacteria. Plates were incubated at 37°C for 72 hours under microaerophilic conditions. Bacterial suspension was prepared in normal saline. The cells were then isolated by centrifugation (9,000\(\times\)g for 10 minutes), and the pellet was used for total RNA extraction. RNA in MDR strains was isolated using the RNA extraction kit (Hoffman-La Roche Ltd.) and then reverse transcribed into cDNA. The hefA vs gyrB (an endogenous gene) was used to study the relative expression of the hefA in 15 MDR clinical strains and strain ST7136 of \(H.\) pylori. cDNA of hefA and gyrB was amplified using RT-PCR (ABI StepOne\(^{TM}\); Thermo Fisher Scientific, Waltham, MA, USA) in the presence of Master Mix (SYBR Green; TaKaRa, Shiga, Japan). The sequences of hefA (GenBank, accession number AF059041), including F: (5′-CTCGCTCGCATGATCGC-3′) and R: (5′-TGATTTGCTTACAA-TTCCCT-3′) were used to amplify a 162-bp fragment. The expression of the housekeeping gene (GenBank, accession number AB084049) was assessed in parallel with the primer pair gyrB: F: (5′-TTACTACGACTTACCTGGGCTAGCGCTG-3′) and R: (5′-CCCATCAATTCCACATTCCGC-3′), amplifying a 267-bp fragment. Then, the hefA expression in MDR and susceptible strain were determined.\(^27\)

**Results**

**Assessment of susceptibilities to antimicrobial agents in clinical strains**

The MIC of Mtz and Cla were determined for 157 strains of \(H.\) pylori. Both biopsy samples and colonies were confirmed by PCR analysis in 22 GC, 38 PUD, and 97 NUD patients by gradient diffusion test. In general, among 157 isolates of \(H.\) pylori, 32 (20.4%) strains were resistant to only one of the antimicrobial agents (Table 1). Single resistance was observed only in Mtz or Cip. The highest resistance rate was attributed to Mtz (n=69, 43.94%). The frequency of double and MDR phenotypes is listed in Table 2. Among the resistant strains, MDR strains were detected in 3 (13.6%), 5 (13.2%), and 7 (7.2%) cases in GC, PUD, and NUD patients, respectively (Table 2).
Hashemi et al.

Genetic variations of the rdxA and frxA in Mtz-resistant strains

The sequences of the rdxA and frxA were analyzed in 57 Mtz-resistant and 5 Mtz-sensitive strains of H. pylori (Figure 1). Sequences of the rdxA and frxA in sensitive strains were pairwise aligned with a reference strain by MEGA version 5 software, showing 95.6%–97.1% and 97.3%–98.7% identity in both genes, respectively. Alteration in amino acid sequences of the rdxA was detected in 50/57 (87.7%) Mtz-resistant strains. Out of 50 strains, 28 (49.1%) translational frameshifts were caused by the presence of premature stop codon through substitution of amino acid in the truncated rdxA (Table 3). The rdxA mutations, including missense and nucleotide insertion/deletion were detected in 8 (14%) and 14 (24.6%), respectively, in the Mtz-resistant strains. In addition, the premature stop codon (29.8%) was seen as the most common mutation in the frxA in Mzt-resistant strains (Table 3). In the MDR strain, the truncated rdxA and frxA were detected as the most common mutation responsible for Mtz resistance (Table 4).

Genetic variations of the 23S rRNA in Cla-resistant strains

The V domain of 23S rRNA was sequenced in the 38 Cla-resistant strains and compared to the reference genome of H. pylori 26695 exhibiting two-point mutations, including substitution of a single nucleotide A2143G (63.1%) and A2142G (34.2%) in 24 and 13 strains, respectively (Figure 1). Simultaneous double mutations of the A2143G and A2142G have not been found in these strains. The A2142C, A2144T, C2245T, G2141A, and G2224A were among the other mutations that have been found in Cla-resistant strains of H. pylori (Table 3).

Expression of efflux pump hefA gene in MDR strains

The expression of hefA and gyrB in MDR and susceptible strain was assessed by RT-PCR assay. Each relative expression value was the mean of three replicates. In 15 clinical isolates, the relative expression of hefA vs gyrB in MDR strains of H. pylori (mean 3.706) was higher than the seven susceptible strains (mean 1.07) (Table 5).

Discussion

In Iran, like other developing countries, H. pylori has infected the majority of the adult population. According to serology data, the incidence of H. pylori infection in Iranian adults is up to 80%.28 In the present study, resistance to Mtz, Cla, Tet, Amx, and Cip were determined in strains of H. pylori by gradient diffusion test (E-test, MAST). Single resistance

Table 1 Prevalence of antimicrobial resistance in clinical Helicobacter pylori isolates

| Antibiotic | MIC in resistant strain (µg/mL) | GC (22), n (%) | PUD (38), n (%) | NUD (97), n (%) | Total, n (%) | P-value |
|------------|---------------------------------|---------------|----------------|----------------|--------------|---------|
| Mtz        | ≥8.0                            | 12 (54.5)     | 15 (39.5)      | 42 (43.3)      | 69 (43.9)    | 0.542   |
| Cla        | ≥1.0                            | 7 (31.8)      | 8 (21)         | 23 (23.7)      | 38 (24.2)    | 0.118   |
| Amx        | ≥2.0                            | 4 (18.2)      | 6 (15.8)       | 13 (13.4)      | 23 (14.6)    | 0.321   |
| Tet        | ≥4.0                            | 6 (27.3)      | 7 (18.4)       | 19 (19.6)      | 32 (20.4)    | 0.177   |
| Cip        | ≥1.0                            | 5 (22.7)      | 6 (15.8)       | 23 (23.7)      | 34 (21.7)    | 0.215   |

Table 2 Primary and combined resistance of Helicobacter pylori isolates to antimicrobial agents

| Antibiotic phenotypes (single, double, triple, and quadruple drug resistance) | GC N=22 | PUD N=38 | NUD N=97 | Total resistant |
|-------------------------------------------------------------------------------|---------|----------|----------|----------------|
| Mtz                                                                           | 4 (18.2)| 6 (15.8) | 17 (14.4)| 27 (17.2)      |
| Cla                                                                           | 5 (22.7)| 6 (15.8) | 17 (14.4)| 38 (24.2)      |
| Amx                                                                           | 7 (30.4)| 8 (21)   | 23 (23.7)| 38 (24.2)      |
| Tet                                                                           | 9 (38.1)| 10 (26.3)| 22 (21.8)| 31 (19.1)      |
| Mtx + Cla                                                                     | 10 (41.3)| 12 (31.6)| 22 (22.7)| 44 (27.3)      |
| Mtx + Cla + Amx                                                               | 12 (50.0)| 14 (36.8)| 26 (26.8)| 52 (32.3)      |
| Mtx + Cla + Tet                                                               | 14 (58.3)| 16 (42.1)| 26 (26.8)| 56 (35.1)      |
| Mtx + Cla + Cip                                                              | 16 (66.7)| 18 (47.4)| 34 (34.4)| 68 (43.1)      |
| Mtx + Cip                                                                     | 18 (75.0)| 20 (52.6)| 38 (38.7)| 76 (48.1)      |
| Total resistant                                                               | 20 (83.3)| 22 (58.9)| 40 (40.8)| 82 (51.8)      |

Abbreviations: Amx, amoxicillin; Cip, ciprofloxacin; Cla, clarithromycin; GC, gastric cancer; Mtz, metronidazole; NUD, non-ulcer dyspepsia; PUD, peptic ulcer disease; Tet, tetracycline.

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was observed only in two antibiotics; Mtz (17.2%) and Cip (3.2%), while double resistance and MDR were seen in 11.5% and 20.4%, respectively. Among 157 H. pylori isolates, 82 (52.2%) cases were resistant to at least one antibiotic, and the highest resistance rate was attributed to Mtz (n=69, 43.9%). The prevalence of resistance to Mtz in strains of H. pylori in Iran is high; the prescription of Mtz as a common anti-parasite drug and as a drug for gynecological disorders could be the cause of the high level of resistance in strains of H. pylori.\textsuperscript{29–32}

In addition, in the mini-review article published by Khademi et al\textsuperscript{3} in Iran, drug resistance data from different strains of H. pylori were collected from different parts of Iran, and the prevalence of Mtz resistance was reported as 61.6%\textsuperscript{30}.

In the literature, limited information exists regarding the mechanism of resistance to Mtz in H. pylori and has not yet been clearly understood. However, some factors that might be relevant to the mechanism of Mtz resistance are as follows: 1) overexpression of TolC homologous genes that upregulates efflux pump activity and reduces activity of nitroreductases and 2) mutational inactivation of the rdxA and/or frxA encoding an oxygen-insensitive NADPH nitroreductase and NAD(P)H flavin oxidoreductase, respectively\textsuperscript{32,33}. These mutations, without leading to death, could be the cause of drug resistance during the clinical application.\textsuperscript{34} Therefore, in agreement with previous studies we have confirmed that Mtz should not be the choice for the first line H. pylori eradication therapy.\textsuperscript{35,36}

In the present study, among Mtz-resistant strains, 63 (92.7%) cases possessed mutation in one or both of the rdxA and frxA, and the most common mutations were due to a premature stop codon (substitution of single amino acid) in the rdxA (40.6%) and frxA (24.6%), while 8.2% of Mtz-resistant strains did not contain any alteration in any of the rdxA and frxA. In the study conducted by Abdollahi et al in Iran, among 35 resistant H. pylori isolates only 22.9% (8/35 isolates) deletion mutations were detected in the rdxA, while in the study performed by Teh et al,\textsuperscript{25} 89.1% of Mtz-resistant strains possessed a premature termination codon or insertion/deletion at one or both of the rdxA and frxA.\textsuperscript{25,37} Therefore, screening for alteration in the rdxA and frxA could be appropriate to identify about 92% of Mtz-resistant strains of H. pylori in the local population.

Resistance to Cla is contributed to the failure of H. pylori eradication in developing countries.\textsuperscript{39} In the present study, the Cla resistance was detected in 24.2% (38/157) of isolates, and was much lower than the resistance to Mtz. In the mini-review article that was published by Khademi et al,\textsuperscript{1} the Cla resistance in strains of H. pylori was detected in 22.4% of

| Patients (resistant) | Cla resistant | Mtz resistant | 23S rRNA mutation, n (%) | FrxA mutation, n (%) | RdxA mutation, n (%) |
|---------------------|---------------|---------------|--------------------------|----------------------|----------------------|
| PUD, n=8            | 1 (12.5)      | 1 (12.5)      | 1 (12.5)                 | 1 (12.5)             | 1 (12.5)             |
| NUD, n=23           | 1 (4.3)       | 1 (4.3)       | 1 (4.3)                  | 1 (4.3)              | 1 (4.3)              |

**Table 3 Mutations detected in the rdxA, fxA, and 23 S rRNA in Mtz and Cla resistant Helicobacter pylori isolated from gastroduodenal disorders.**

**Abbreviations:** Cla, clarithromycin; GC, gastric cancer; Mtz, metronidazole; NUD, non-ulcer dyspepsia; PUD, peptic ulcer disease.
isolates, while the low resistance to Cla was reported from neighboring Asian countries, including Egypt (4%), Israel (8.2%), Saudi Arabia (4%), and Kuwait (0%).30,38,39 Therefore, the rate of Cla-resistant strains of H. pylori, in Iran is higher than in the Middle East and developed countries. The three most frequent point mutations, the A2143G, A2142G, and A2142C in the 23S rRNA component of ribosomes were related to Cla resistances in H. pylori.40–42 In the present study, the obtained sequences were aligned with the reference strain 26695 of H. pylori, and out of 38 Cla-resistant strains, 37 (97.3%) cases had mutations, such as the A2143G and A2142G. The A2143G (63.1%) was the most common point mutation in the 23S rRNA, followed by 34.2% of the A2142G substitution. There was no point mutation in the sensitive strains of H. pylori. Other mutations that have been observed in Cla-resistant H. pylori isolates were the A2142C and G2141A, G2224A, G2144T, and C2245T. Therefore, Cla-resistant strains could be detected in 97.3% (37/38) of the local population, by screening for mutations in conserved V domain of genes, encoding the 23S rRNA. In the study conducted by Teh et al,25 V domain of the 23S rRNA was sequenced in 14 Cla-resistant strains, and by screening for the A2142G or A2143G mutations of the 23S rRNA, they were able to detect 92.9% of Cla-resistant strains in the Malaysian population.25

The uncontrolled use of antibiotics may have led to the emergence of MDR strains of H. pylori. In the present study, MDR strains were detected in 9.6% (15/157) of H. pylori isolates. The truncated rdxA (caused by premature stop codon) and deletion/insertion were detected as the most common mutational inactivation in the rdxA and frxA. Double point mutations were only detected in two MDR strains, including the ST3204: A2143G, G2224A and ST3188: A2143G,

Table 4  Mutation patterns of Mtz- and Cla-resistant strains in MDR Helicobacter pylori strains

| MDR strain | MIC (µg/mL) | Mutation description in Cla | Mutation description in Mtz | rdxA |
|------------|-------------|-----------------------------|-----------------------------|------|
| ST3175     | 32          | 64                          | A2143G                      | Premature stop codon at Arg86 |  |
| ST3179     | 4           | 32                          | A2142G                      | Premature stop codon at Gin5 |  |
| ST3177     | 8           | 256                         | A2143G                      | Deletion of 3 nt (38–40)      |  |
| ST3188     | 16          | 256                         | A2143G, C2245T              | Premature stop codon at Met66 | Deletion of 3 nt (38–40) |
| ST3182     | 32          | 64                          | A2142G                      | –                           | M56V |
| ST3187     | 8           | 128                         | A2143G                      | L62V                         | – |
| ST3183     | 4           | 32                          | A2143G                      | –                           | V32A |
| ST3188     | 8           | 16                          | A2142G                      | Deletion of 3 nt (84–86)      | – |
| ST3191     | 32          | 64                          | A2142G                      | Premature stop codon at Trp137 | – |
| ST3196     | 32          | 32                          | A2143G                      | Deletion of 2 nt (83–84)      | Insertion of 3 nt (80–82) |
| ST3201     | 8           | 32                          | A2142G                      | –                           | Premature stop codon at Arg16 |
| ST3203     | 4           | 128                         | A2143G, G2224A              | Insertion of 1 nt (72)        | Deletion of 3 nt (151–153) |
| ST3204     | 16          | 32                          | A2143G                      | Premature stop codon at Tyr60 | S111L |
| ST3205     | 8           | 128                         | A2142C                      | –                           | Premature stop codon at Gly189 |
| ST3194     | 32          | 128                         | A2143G                      | –                           | – |

Abbreviations: Cla, clarithromycin; Mtz, metronidazole; MDR, multidrug-resistant; MIC, minimum inhibitory concentration; nt, nucleotide.

Table 5  Relative expression of hefA gene vs gyrB in MDR clinical strains compared to susceptible strain

| Strain          | Sequence type | hefA/gyrA |
|-----------------|---------------|-----------|
| MDR strain (n=15) |               |           |
| ST3175          | 4.47          |           |
| ST3179          | 3.95          |           |
| ST3177          | 7.25          |           |
| ST3188          | 3.12          |           |
| ST3182          | 4.11          |           |
| ST3187          | 3.94          |           |
| ST3183          | 3.12          |           |
| ST3188          | 4.15          |           |
| ST3191          | 2.27          |           |
| ST3196          | 3.07          |           |
| ST3201          | 2.62          |           |
| ST3203          | 3.32          |           |
| ST3204          | 2.48          |           |
| ST3205          | 3.98          |           |
| ST3194          | 3.74          |           |
| Mean            | 3.706         |           |
| Susceptible strain (n=7) |         |           |
| ST3202          | 0.95          |           |
| ST3192          | 1.02          |           |
| ST3195          | 0.87          |           |
| ST3189          | 0.92          |           |
| ST3186          | 1.21          |           |
| ST3176          | 1.13          |           |
| ST3178          | 0.97          |           |
| Mean            | 1.01          |           |

Abbreviation: MDR, multidrug resistant.
Figure 1 Distribution of included patients and genetic mutations related with Mtz and Cla resistance in Helicobacter pylori strains isolated from gastroduodenal disorders. 

Notes: (A) Prevalence of gastroduodenal disorders in males and females. Mutations in rdxA, frxA, and 23S rRNA in (B) GC, (C) PUD, and (D) NUD.

Abbreviations: Cla, clarithromycin; GC, gastric cancer; Mtz, metronidazole; NUD, non-ulcer dyspepsia; PUD, peptic ulcer disease.
C2245T. Overall, no correlation was found between levels of MIC between Cla resistance and the point mutation in MDR strains.

Our findings suggested that the efflux pump system was overexpressed in the MDR strains of *H. pylori*, suggesting that the long-term and inappropriate administration of antibiotics in the clinic may be responsible for the appearance of these strains. Chromosomally encoded drug resistances in *H. pylori* via overexpression of the efflux pump systems were associated with MDR to antibiotics. In the present study, based on RT-PCR, our findings suggested that the efflux pump *hefA* were overexpressed in the MDR strains of *H. pylori*. The relative gene expression of *hefA* vs *gyrB* in 80% (12/15) of clinical MDR strains of *H. pylori* was significantly higher (*P*<0.05) in comparison to five susceptible strains. In general, antimicrobial susceptibilities of the isolates collected from GC, PUD, and NUD patients were compared, and no significant difference (*P*>0.05) in antimicrobial resistance was observed among these groups.

In spite of these observations, a major limitation of this study is the genome diversity of *H. pylori*. The main cause of resistance to Mtz and Cla is point mutations, which could influence efflux pump-related antibiotic resistance patterns. Therefore, it is difficult to distinguish between these two antibiotic resistance mechanisms. In conclusion, *H. pylori* genome evolves and shows wide geographical divergence. The prevalence of resistance to *H. pylori* antimicrobial agent is rapidly growing in Iran, and MDR strains have recently been reported. Mutational inactivation and the overexpression of efflux pump are two mechanisms that increase the resistance to *H. pylori* antimicrobial agents and the rate of MDR strains. In Iran, the mutations of *rdxA* and *frxA* in Mtz-resistant strains and the A2143G and A2142G mutations of 23S rRNA in Cla-resistant strains were significant, and screening for these mutations could help to determine the most effective anti-*H. pylori* drugs and to prevent antibiotic resistance.

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**Author contributions**

All authors contributed toward data analysis, drafting and revising the paper, gave final approval of the version to be published and agree to be accountable for all aspects of the work.

**Disclosure**

The authors report no conflicts of interest in this work.

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