RESEARCH ARTICLE

Identification of a Novel Cassette Array in Integron-bearing *Helicobacter Pylori* Strains Isolated from Iranian Patients

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Abstract

Helicobacter pylori **as the second most common cause of gastric cancer in the world infects approximately half of the developed countries population and 80% of the population living in developing countries. Integrons as genetic reservoirs play major roles in dissemination of antimicrobial resistance genes. To the best of our knowledge, this is the first study to report carriage of class 1 and 2 integrons and associated gene cassettes in *H. pylori* isolates from Iran. This cross-sectional study was conducted in Tehran among 110 patients with *H. pylori* infection. Antimicrobial susceptibility testing (AST) for *H. pylori* strains were assessed by the micro broth dilution method. Class 1 and 2 integrons were detected using PCR. In order to determine gene cassettes, amplified fragments were subjected to DNA sequencing of both amplicon strands. The prevalence of resistance to clarithromycin, metronidazole, clarithromycin, tetracycline, amoxicillin, rifampin, and levofloxacin were 68.2% (n=75), 25.5% (n=28), 24.5% (n=27), 19.1% (n=21), 18.2% (n=20) and 16.4% (n=18), respectively. Frequency of multidrug resistance among *H. pylori* isolates was 12.7%. Class 2 integron was detected in 50 (45.5%) and class 1 integron in 10 (9.1%) *H. pylori* isolates. The most predominant gene cassette arrays in class 2 integron-bearing *H. pylori* were included sat-era-aadA1, dfrA1-sat2-aadA1, blaoxa2 and, aadB whereas common gene cassette arrays in class 1 integron were aadB-aadA1-cmlA6, aacA4, blaoxa2, and catB3. The high frequency of class 2 integron and multidrug resistance in the present study should be considered as a warning for clinicians that continuous surveillance is necessary to prevent the further spread of resistant isolates.**

Keywords: ***H. pylori*** - integron s- multidrug-resistant strains - Iran

Asian Pac J Cancer Prev, **17 (7)**, 3309-3315

Introduction

Helicobacter pylori (*H. pylori*) is a curved gram-negative, rod-shaped, flagellate, microaerophilic spiral bacillus and presently classified as a group 1 carcinogen by the World Health Organization International Agency for Research on Cancer (WHO/IARC). *H. pylori* plays an important role in chronic gastritis, peptic ulcers, gastric lymphoma and the development of adenocarcinoma (Malfertheiner et al., 2012). Gastric cancer is the second most common cancer in the world while long-standing infection with *H. pylori* significantly increases the risk of developing this disease.

H. pylori as one of the most common chronic bacterial infections worldwide is colonized in the stomachs of about 50-60% of the world's population. Bacterial infection has been spread over approximately 50% of the developed countries and 80% of the population living in developing countries (Correa and Piazuelo, 2008). The prevalence of *H. pylori* infection varies globally in different populations and is associated with geographic area, socioeconomic factors, personal hygiene and age. *H. pylori* infection is generally acquired in childhood (Rafeey et al., 2007). Treatment of *H. pylori* infection is recommended in all symptomatic individuals, which is the main factor for eradication of *H. pylori* infection. Based on previous reports, *H. pylori* eradication leads to the reduction of the severity of gastric disease symptoms, the development of atrophic gastritis, the risk of cancer progression and complete recovery of patients (Smith et al., 2014).

Widespread antibiotics use for killing or eradication of enteric pathogens and respiratory tract infections led to the emergence of MDR to *H. pylori*. Failure of therapy not only leads to worsening of disease but also increases the resistance of the bacterium to the prescribed antibiotics (Rafeey et al., 2007; Smith et al., 2014).

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Increase of resistance to therapeutic option of *H. pylori* infection is the most serious problem in eradicating *H. pylori*. Unfortunately, it is documented that in Iran and lots of other developing countries, the eradication rate of *H. pylori* is much lower than the rate reported in other countries (Milani et al., 2012; Fathi et al., 2013). Considering extreme genetic heterogeneity of *H. pylori*, several mechanisms of resistance to antibiotics are accurately detailed (point mutations, redox intracellular potential, pump efflux systems, membrane permeability and penetration of bacterium in the mucous layer of stomach) (Nishizawa and Suzuki, 2014). Recently, horizontal gene transfer mediated by mobile genetic elements, e.g., plasmids, transposons and integrons, have been shown to contribute to the spread of antibiotic resistance genes among bacteria (Crespo et al., 2005). Integrons as a motionless genetic element which consists of 5´and 3´-conserved segments with gene cassettes containing antibiotic resistance genes are major elements in the spread of MDR particularly in Gram-negative species (Gillings, 2014). The basic structure of an integron consists of an integrase gene (intI) encoding an integrase, a recombination site (attI) and a strong promoter (PC) gene that permits the expression of the gene cassettes. To date, several classes of integrons have been described that are recognized by their distinct integrase genes. Class 1 and 2 integrons are the most common and widely distributed among gram-negative bacteria isolated from clinical samples. Integron gene sequences contribute to the spread of antimicrobial resistance alleles by lateral gene transfer. Class 2 integrons are embedded in Tn7, and are less common than class 1 integrons. Other classes of integrons were rarely reported (Cambray et al., 2010; Gillings, 2014). According to the literature reviews, the prevalence of *H. pylori* infection is steadily increasing in Iran (Rafeey et al., 2007; Milani et al., 2012). In the present research, dissemination of different classes of integrons and their gene cassettes in *H. pylori* has been studied for the first time in Iran. We also assessed susceptibility of *H. pylori* isolates to the commonly used antibiotics for eradication regimens.

Materials and Methods

Sampling and data collection

This cross-sectional study was conducted on 154 biopsy specimens obtained from patients with suspected *H. pylori* infection who were referred to gastroenterology wards of Tehran (capital city of Iran) hospitals during a 9-month period from 1 January 2015 to the end of September 2015. None of the participants in this study had a history of use of non-steroidal anti-inflammatory drugs (NSAIDs) and proton pomp inhibitors in three weeks before proceeding to endoscopy. Two gastric biopsy specimens were taken from each patient. One of them was used for rapid urease test and the other was placed in sterile tubes containing 10 ml Stuart medium (Merck, Germany) and transported to the microbiology laboratory within 4 h of collection. For bacterial identification, homogenized gastric biopsy samples were cultured in Brucella blood agar (Merck, Germany) containing defibrinated sheep blood (5%) and antibiotic supplements (vancomycin 5 mg/L, trimethoprim 5 mg/L, and polymyxin B 0.25 mg/L). The plates were incubated under microaerophilic conditions (5% O2, 10% CO_2, 85% N2) at 37°C and high humidity for 3 to 7 days. Organisms were identified as *H. pylori* based on gram stain, culture, oxidase, catalase and rapid urease test. Samples confirmed as *H. pylori* isolates were stored in Brain Heart Infusion broth containing (BHI; Merck, Germany) containing 30% glycerol at -70°C for further studies. The study protocol was approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences (No. 381).

Antimicrobial susceptibility testing

Pure cultures of *H. pylori* isolates were used for antibiotic susceptibility test. Antimicrobial susceptibility of *H. pylori* isolates was assessed by minimum inhibitory concentration (MIC) test according to the guidelines of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (www.eucast.org). The following antimicrobial agents were purchased from Sigma-Aldrich (St. Louis, Mo) and used in this study: metronidazole, levofloxacin, clarithromycin, amoxicillin, rifampicin and tetracycline. Minimum inhibitory concentration (MIC) was defined as the lowest concentration of each antimicrobial agent that inhibited visible growth of the tested isolate. The ranges of MIC value used for antimicrobial agents were including: metronidazole 0.25 to 32 μg/ml; rifampicin 0.25 to 8 μg/ml; clarithromycin 0.125 to 4 μg/ml; amoxicillin 0.125 to 16; levofloxacin 0.125 to 4 μg/ml; tetracycline 0.125 to 16 μg/ml. *H. pylori* ATCC 26695 strain was used as the quality-control organisms in antimicrobial susceptibility determination. MIC_{50} and MIC_90 (MICs that inhibit 50% and 90% of the isolates) were estimated for each antibiotic.

Extraction of plasmid and genomic DNA

Genomic DNA was extracted using the QIAamp DNA isolation columns (Qiagen, Hilden, Germany) according to the manufacturer's procedure. The Qiagen Plasmid Midi Kit was used for plasmid DNA extraction according to the manufacturer's instruction. The concentration of DNA was assessed by spectrophotometer. DNA was stored at -20°C for further studies.

Detection of integrons and gene cassettes inserted in the variable regions

Detection of class 1 and 2 integrons was carried out by PCR using degenerate primer sets described by Moura et al. which hybridizes to conserve regions of integron-encoded genes (Moura et al., 2007). Amplified fragments of the class 1 and 2 integrons were 280bp and 232bp, respectively. PCR was done by thermocycler (Eppendorf, Hamburg, Germany) using the following modified conditions; initial denaturation for 4 min at 94ºC, 35 cycles of denaturing at 94ºC for 1 min, annealing at 57ºC for 45 s, and extension at 72ºC for 45 s. The final extension was carried out at 72ºC for 5 min.

Amplification of the variable region between class 1 and 2 integrons was performed using degenerated primer pairs described by Moura et al., (Moura et al., 2007).

PCR products of variable regions were sequenced after purification using QIAquick Gel Extraction kit (Qiagen, Hilden, Germany) according to the manufacturer's instruction. Purified PCR products of variable regions were sequenced by an ABI Prism 377 automated sequencer (Applied Biosystems, Perkin-Elmer, Foster City, CA) in both directions. The sequences were assembled using SeqMan program within the Lasergene suite version 7 (DNAstar Inc, Madison, WI, USA). The BLAST program (http://blast.ncbi.nlm.nih.gov/Blast.cgi) was performed repeatedly for sequence comparison and annotation.

Statistical analysis

Statistical analysis was performed using the SPSS version 18.0 software (SPSS Inc., Chicago, IL). Categorical variables were analyzed by Chi-square test. A P value ≤ 0.05 was statically significant.

Results

During the 9-months study period, a total of 154 biopsy specimens were taken from patients with suspected *H. pylori* infection and a total of 110 samples (71.4%) were *H. pylori* positive which were entered to the study. In this study, 40 (36.4%) patients were females and 70 (63.6%) patients were males (age range 34-70 years). Out of 110 patients entered in the study who were screened, 45 (40.9%) had gastric cancer, 37 (33.6%) duodenal ulcer, 12 (10.9%) peptic ulcer, 10 (9.1%) gastric ulcer and 6 (5.5%) gastric cancer with peptic ulcer. *H. pylori* resistance rates to antimicrobial agents were as follows: metronidazole 68.2% (n=75), clarithromycin 25.5% (n=28), tetracycline 24.5% (n=27), amoxicillin 19.1% (n=21), rifampicin 18.2% (n=20) and levofloxacin 16.4% (n=18). Out of 75 isolates resistant to metronidazole, 25 (33.4%) of isolates had MIC > 8μg/ml, 10 (13.3%) had MIC ≥ 16μg/ml, 30 (40%) had MIC ≥ 32μg/ml and 10 (13.3%) had MIC ≥ 64μg/ml. Of 10 isolates resistant to metronidazole with MIC ≥ 64μg/ml, 6 isolates were isolated from patients with gastric cancer and peptic ulcer and 4 isolates were isolated from patients with gastric cancer. All of the metronidazole-resistant strains with MIC ≥ 32μg/ml except for seven isolates were isolated from patients with gastric cancer. The MIC values of metronidazole for the rest of isolates (31.8%, n=35) were ranged from 0.5 to 4 μg/ml. The results of clarithromycin MIC were as follows: 26 (23.6%) of isolates had MIC 0.125 μg/ml, 56 (51%) had MIC 0.25 μg/ml, 3 (2.7%) had MIC 0. 5 μg/ml, 10 (9.1%) had MIC 1 μg/ml, 4 (3.6%) had MIC 1 μg/ml, 5 (4.5%) had MIC 2 μg/ml and 6 (5.5%) had MIC 4 μg/ml. Of six resistant isolates to clarithromycin with MIC ≥ 4μg/ml, three isolates were isolated from patients with peptic ulcer and the remaining three isolates were obtained from patients with gastric ulcer. As previously mentioned, 24.5% of the isolates were resistant to tetracycline. Out of 27 resistance isolates to tetracycline, 20 (74.1%) of isolates had MIC ≥ 2 μg/ml and 7 (25.9%) had MIC ≥ 4 μg/ml. All susceptible isolates except two of them were inhibited by tetracycline at MIC ≤ 0.5μg/ml. In our survey, 19.1% of the isolates were resistant to amoxicillin (MIC ≥ 2 μg/ml). Out of 21 resistance isolates to amoxicillin, 20 (95.2%) of isolates had MIC ≥ 2 μg/ml, whereas, only one isolate (4.8%) had MIC ≥ 4 μg/ml. Out of 21 isolates resistant to amoxicillin, one isolate was isolated from patient with gastric cancer, 15 isolates from patients with duodenal ulcer and 5 isolates from patients with gastric ulcer. All of the *H. pylori* isolates resistant to rifampicin except five isolates (three isolates had MICs ≥ 2μg/ml and the remaining two isolates had MIC > 1 μg/ml) were inhibited at MIC ≥ 4 μg/ml. The MIC values of rifampicin for remaining 90 (81.8%) of isolates was ranged from 0.125 to 1 μg/ml. Out of 20 isolates resistant to rifampicin, 15 isolates were isolated from patients with gastric cancer, three isolates from patients with duodenal ulcer and two isolates from patients with gastric ulcer. The lowest level of resistance was related to levofloxacin (16.4%). Out of 18 resistance isolates to levofloxacin, 12 (66.7%) of isolates had MIC ≥ 2 μg/ml and six (33.3%) had MIC ≥ 4 μg/ml. Isolates with resistance to levofloxacin were isolated from patients with gastric cancer (55.5%), duodenal ulcer (27.8%) and peptic ulcer (16.7%). MDR was defined as resistance to three or more antibiotics of different classes (Boyanova et al., 2015, http://dx.doi.org/10.1016/j.ijantimicag.2015.08.010). Frequency of MDR in our survey was 12.7%. In particular, 68 (61.8%), 12 (10.9%) and two isolates (1.8%) were resistant to two, three and four different drugs, respectively.

The predominant resistance profile among the studied isolates was included resistance to metronidazole and tetracycline. Pattern of multiple resistances of 110 *H. pylori* isolates to different antibiotics are showed in Table 1.

Integron was detected in 64 isolates (58.2%). PCR results for detection of class 1 and class 2 integrons

Table 1. Frequency of Multiple Resistance of *H. pylori* isolates to Antimicrobial Agents

Resistance pattern	No (%)
MTZa+TET[b]	25(22.7)
MTZa+LEV[c]	18(16.4)
MTZa+CLM[d]	15(13.6)
MTZa+AMX[e]	10(9.1)
MTZa+CLMd+RIF[f]	5(4.5)
MTZa+AMXe+CLM[d]	7(6.3)
MTZa+AMXe+TETb+RIF[f]	2(1.8)

[a] Metronidazole; [b] Tetracycline; [c] levofloxacin; [d] clarithromycin; [e] amoxicillin; [f] rifampicin

Table 2. Frequency of Integron in 110 *H. pylori* Clinical Isolates

Integron class	Frequency (%)
Integron class 1	10(9.1%)
Integron class 2	50(45.5%)
Integron class 1 and 2	4(3.6%)
Integron class 3	0(0)
Without integron	46(41.8)
Total	110(100)

Table 3. Antibiotic Resistance and Integrons in *H. pylori* Clinical Isolates

Antibiotics	MIC(μg/ml)			Antibiotic susceptibility (n=110)		Integron positive(n=64)		Inetgron negative(n=46)	
	Range	50%	90%	R	S	R	S	R	S
				n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
metronidazole	0. 25-32	0.5	2	75(68.2)	35(31.8)	63(98.4)	1 (1.6)	12(26.1)	34(73.9)
clarithromycin	0.125-4	1	2	28(25.5)	82(74.5)	14(21.9)	50(78.1)	14(30.4)	32(69.6)
tetracycline	0.125-16	1	1	27(24.5)	83(75.5)	21(32.8)	43(67.2)	6(13)	40(87)
amoxicillin	v	0.5	0.5	21(19.1)	89(80.9)	20(31.3)	44(68.7)	1(2.2)	45(97.8)
rifampicin	0.125-4	0.5	0.5	20(18.2)	90(81.8)	15(23.4)	49(76.6)	5(10.9)	41(89.1)
levofloxacin	0.5-16	0.5	1	18(16.4)	92(83.6)	11(17.2)	53(82.8)	7(15.2)	39(84.8)

revealed a dominant existence of class 2 integron in 50 (45.5%) *H. pylori* isolates, whereas class 1 integron was detected in 10 isolates (9.1%). Also, co-existence of class 1 and 2 integrons was detected in four isolates (3.6%). Frequency of integrons in 110 *H. pylori* clinical isolates are shown in Table 2. Out of 50 isolates carrying class 2 integron, 28 isolates (56%) were obtained from patients with gastric cancer, 10 isolates (20%) from with duodenal ulcer patients, five isolates (10%) from patients with peptic ulcer, four isolates (8%) from patients with gastric ulcer three isolates (6%) from patients with gastric cancer and peptic ulcer. All class 1 integrons bearing isolates were isolated from patients with gastric cancer. Class 2 integrons in 45 (90%) isolates were located on plasmid and in five (10%) isolates were located on chromosome while integron class 1 in seven isolates (70%) were located on chromosome and in 3 isolates (30%) were located on plasmid. Integron frequency among resistant and susceptible strains is presented in Table3. Common gene cassettes arrays in class 2 integron included dfrA1-sat2-aadA1, Sat-era-aadA1, blaoxa2 and, aadB whereas common gene cassettes arrays in class 1 integron included aadB-aadA1-cmlA6, aacA4, blaoxa2, and catB3. dfrA1-sat2-aadA1 gene cassette was detected in 13 integron class 2-bearing *H. pylori* strains, Sat-era-aadA1 as a novel gene cassette in 12 strains, and blaoxa2 gene cassette (confer resistance to oxacillin and ampicillin) in 12 strains. aadB gene cassette (confer resistance to gentamicin, tobramycin, and kanamycin) was detected in 10 class 2 integron-bearing *H. pylori* strains. Three isolates of class 2 integron-positive harbored no gene cassette. aadB-aadA1-cmlA6 was detected in 5 integron class 1-bearing *H. pylori* strains (aadA1 confer resistance to streptomycin and spectinomycin), aacA4 gene cassette in 2 class 1 integron -bearing *H. pylori* strains (confer resistance to gentamicin, amikacin and tobramycin resistance) and catB3 gene cassette in 3 class 1 integron -bearing *H. pylori* strains (confer resistance to chloramphenicol). All the isolates with integron class 1 harbored gene cassette.

Nucleotide sequence accession number

The nucleotide sequence of new cassette array obtained from in this study is available in the GenBank nucleotide database under accession numbers LC095818. It is also available in Integron Database INTEGRALL (http://integrall.bio.ua.pt/).

Discussion

In the current study, prevalences of class 1 and 2 integrons were examined among multidrug resistant *H. pylori*, isolated from 110 patients. The prevalence of antimicrobial-resistant *H. pylori* infection is of considerable public health concern especially in developing countries (Milani et al., 2012; Fathi et al., 2013).

Effective therapy plays an important role in the eradication of *H. pylori*-associated disorders. Eradication treatment for *H. pylori* infection requires to combination therapy, consisting of one or two antibiotics (amoxicillin, metronidazole or clarithromycin) with an acid suppressor (usually a proton pump inhibitor) or a histamine receptor (H2-receptor) antagonist (e.g. ranitidine) (Correa and Piazuelo, 2008; Malfertheiner et al., 2012). According to the literature, proper regimen along with improved public knowledge of personal hygiene can lead to 85–90% eradication rate. Inappropriate use of antimicrobial agents, limited therapeutic options, and also emergence of MDR strains of *H. pylori* are considered a major factor contributing to treatment failure (Fuccio et al., 2009). Therefore, consciousness about *H. pylori* antibiotic resistance pattern is necessary to prescribe the most effective therapy regimen and prevent further spread of resistant isolates. Antibiotic resistance pattern of *H. pylori* to antimicrobial agents varies in different geographical regions and depends on local use of antibiotics (Milani et al., 2012).

According to the literature, the rate of resistance to metronidazole in *H. pylori* that has been reported to range between eight and 80% in different countries, varies according to geographical region (Nishizawa and Suzuki, 2014). Our results showed a very high resistant rate to metronidazole (68.2%) among isolates, which were significantly higher in female than in male patients. In Loffeld et al. (Loffeld and Werdmuller, 2013) study in Netherland, antibiotic susceptibility of *H. pylori* in native Dutch patients and patients of Turkish descent was determined. They investigated 925 strains of *H. pylori* during a period of eight years. They showed resistance to metronidazole in 147 isolates (19.9%). In another study conducted in Sofia, Bulgaria in 2015, Boyanova et al. evaluated the susceptibility of 53 *H. pylori* strains by Etest. Resistance to metronidazole was reported in 37.7% of isolates (Boyanova et al., 2015, http://dx.doi.org/10.1016/j.ijantimicag.2015.08.010). Goudarzi et al. in a study conducted in Iran in 2014, showed that highest levels of resistance was related to metronidazole (66.3%) (Goudarzi et al., 2015). In Turkey, the rate of resistance to metronidazole reported in 35.5% (34/98) of clinical strains of *H. pylori* (Caliskan et al., 2015). Manfredi et al. assessed

the antimicrobial susceptibility changes in children with *H. pylori* infection in Italy over a 13-year period from 1998 to 2012. They showed reduction in metronidazole resistance from 56% in 1998/99 to 33% in 2011/12 (Manfredi et al., 2015). High resistance to metronidazole among *H. pylori* clinical isolates has been reported from Iran and some other Asian countries. These findings about resistance rate to metronidazole in our study were consistent with some reports from developing countries that described a high level of resistance to metronidazole, which varies from 66.2% to 100% (Sherif et al., 2004; Aboderin et al., 2007). Overall, in developed countries it has been documented that 15.8% to 40% of *H. pylori* strains were resistant to metronidazole (Parsons et al., 2001; Boyanova et al., 2002). These discrepancies in the rate of resistance to metronidazole in our study compared to other studies might be explained by the extensive use of this antibiotic in treatment of diseases caused by *H. pylori* and common different infections such as protozoal diseases, and periodontal or gynaecological infections. However, the role of various gene mutations in NADPH nitroreductase (RdxA), NADPH-flavin-oxidoreductase (FrxA), and ferredoxin likeenzymes (FrxB) should not be ignored in resistance to metronidazole (Masaoka et al., 2006).

Clarithromycin as the first line therapy against *H. pylori* infections and an essential antibiotic for *H. pylori* treatment has an increased resistance rate from 9% in 1998 to 17.6% in 2008 in Europe (Asaka et al., 2010). The second most resistance, which was identified in our study, was resistance to clarithromycin. Of the 110 *H. pylori* isolates, 28 (25.5%) showed resistance to clarithromycin. These results are in accordance with a previous study done in Tehran, Iran in 2014, by Goudarzi et al. which showed that the rate of resistance to clarithromycin was 18.4% (Goudarzi et al., 2015). Furthermore, Loffeld et al. reported a 20.5% resistance rate to clarithromycin in their tested isolates (Loffeld and Werdmuller, 2013). Overall, compared to performed studies in Bulgaria (Boyanova et al., 2015, http://dx.doi.org/10.1016/j.ijantimicag.2015.08.010), Brazil (Ogata et al., 2014), Netherlands (Loffeld and Werdmuller, 2013), Italy (Manfredi et al., 2015), Sweden (1.5%), and Malaysia (2.1%) (De Francesco et al., 2006), a high resistance to clarithromycin was observed in our study. These differences in resistance to clarithromycin in our study with other studies could be attributed to frequent use of macrolides in patients with upper respiratory tract infection, the efflux pump system and point mutations.

Resistance to tetracycline was observed in 24.5% of our isolates which was higher than the reported rates in Korea (0.5%) (Chung et al., 2012), Bulgaria (1.9%) (Boyanova et al., 2015, http://dx.doi.org/10.1016/j.ijantimicag.2015.08.010), Turkey (0%) (Caliskan et al., 2015), Brazil (0%) (Ogata et al., 2014), Germany (0%) (Wueppenhorst et al., 2013) and India (4%) (Ahmed et al., 2012) and lower than the reported rate in Africa (43.9%) (De Francesco et al., 2006). In previous study conducted in Iran, in 2014, Goudarzi et al., found a low resistance rate to tetracycline (3.1%) in *H. pylori* clinical isolates (Goudarzi et al., 2015).

The present study revealed a high resistance rate to tetracycline that might be related to a greater use of this antibiotic in treatment of most of infections, mutations in the 16S rRNA, and horizontal transfers.

In the present survey, we found 21 resistant isolates to amoxicillin out of the 110 tested isolates (19.1%). Based on the most recent data available from the literature, results on amoxicillin resistance are highly contradictory. Amoxicillin resistance has fortunately remained low in several countries. The low and/or no amoxicillin resistance rates are reported in some countries, such as Kuwait (John Albert et al., 2006), Senegal (Seck et al., 2009), Netherlands (Loffeld and Werdmuller, 2013), Bulgaria (Boyanova et al., 2015, http://dx.doi.org/10.1016/j.ijantimicag.2015.08.010), Korea (Kim et al., 2006) and Turkey (Caliskan et al., 2015). The reported rate in the present survey (19.1%) is lower than India (Ahmed et al., 2012), China (Wu et al., 2000), Brazil (Godoy et al., 2003) and Cameroon (Ndip et al., 2008). Reports of Iran also showed a wide difference of amoxicillin resistance rates; for instance the findings of Milani et al.,(Milani et al., 2012) revealed 28.6% resistance to amoxicillin while in the same context, goudarzi et al., (Goudarzi et al., 2015) reported amoxicillin resistance in 5.1% of the strains. This wide variation in amoxicillin resistance rates reported from different countries may be the consequence of misuse and overuse of this antibiotic, point mutation on pbp1, β-lactamase production. The rate of resistance to levofloxacin in the study was relatively low (16.4%). This result was strongly supported by the findings of Bulgaria (Boyanova et al., 2015, http://dx.doi.org/10.1016/j.ijantimicag.2015.08.010), Japan (Kobayashi et al., 2007) and Taiwan (Hu et al., 2007). Although, the rate of resistance to levofloxacin differ in different countries, the results of the present study in comparison with previous study conducted in Iran revealed the rapid emergence of levofloxacin resistance in *H. pylori*.

The present study revealed that 12.7% of isolates were MDR. The incidence of multiple resistance of *H. pylori* isolates to common therapeutic agents was in agreement with Milani et al. (Milani et al., 2012) who reported ten of examined 112 strains (9%) were resistant to four antibiotics and 4 of examined 112 strains (3.6%) were resistant to more than 4 antibiotics. Our study showed a high carrier rate of plasmid (75%) among *H. pylori* harboring integron. This result was supported by the findings of Crespo et al. (Crespo et al., 2005) who believed Tn7 could be probably located in plasmid DNA and confirmed antimicrobial resistance in *H. pylori* strains.

To our knowledge, this is the first study to report carriage of class 1 and 2 integrons and associated gene cassettes in *H. pylori* isolates from Iran. It is noteworthy that a dominant existence of class 2 integron 50 (45.5%) was seen in our *H. pylori* isolates. This finding is in consistent with a study conducted by Crespo et al. in Argentina that revealed a high carrier rate (37.5%) of integron class 2 in clinical isolates of *H. pylori* (Crespo et al., 2005).

A study conducted in China in order to investigate the status of the plasmid and classes 1-3 integrons in metronidazole resistant *H. pylori* strains isolated from a

region at high risk of gastric cancer. Yue et al. (Yue et al., 2014) detected resistance to metronidazole in 88 isolates (51.16%) meanwhile none of isolates carried integron gene even on plasmid. This discrepancy in frequency of integron and gene cassettes in *H. pylori* isolates could be explained by overuse of antibiotics for treatment gastrointestinal disorders, various treatment protocols applied in different geographic regions and the ability of *H. pylori* isolates in horizontal gene transfer among susceptible and resistant strains.

This survey elucidated a high prevalence of antibiotic resistance in *H. pylori* isolates in Iran. According to our findings, metronidazole is not an effective drug for treatment of *H. pylori* infections. Clinicians must consider that guidelines for the management of *H. pylori* infection should be revised. Established therapies should be recommended in order to successful eradication of *H. pylori* infections. Although the main cause of antibiotic resistances in *H. pylori* may be induced by de novo, it can be justified by horizontal gene transfer as well. High frequency of class 2 integron with diverse cassette arrays play a role as a genetic reservoir for the dissemination of antimicrobial resistance gene and could be a severe problem for *H. pylori* treatment.

Acknowledgements

This project was supported financially by the grant (No. 30928) from Research Deputy of Shahid Beheshti University of Medical Sciences. The authors would like to thank the staff of School of Medicine and Paramedicine, Shahid Beheshti University of Medical Sciences. We also thank the individuals and organizations who participated in our research.

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