

Genotyping and antibiotic resistance properties of *Helicobacter pylori* strains isolated from human and animal gastric biopsies

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Purpose: The present study was done to assess the prevalence rate, antibiotic resistance pattern and genotyping status of the *Helicobacter pylori* strains isolated from human and animal gastric biopsy samples.

Patients and methods: A total of 1,150 gastric biopsy samples were randomly collected from humans (children and adults) and animals (cows, sheep and goats). All samples were subjected to culture, urease test and histopathologic examination. *H. pylori* isolates were also confirmed using the *I6S rRNA* gene PCR-amplification. Antibiotic resistance pattern was assessed by the disk diffusion method. Distribution of different genotypes was studied by PCR.

Results: The prevalence of *H. pylori* in gastric biopsy samples which were studied using urease test, culture and histological examination were 57.04%, 55.40% and 60.80%, respectively. Samples that were collected from adult humans (78%) and sheep (70%) had the highest prevalence of *H. pylori* strains, while those of goats (0.6%) and cows (4%) had the lowest. Findings of the culture method were confirmed using PCR-based amplification of *I6S rRNA*. Distribution of *H. pylori* among the gastric ulcers, duodenal ulcers, chronic gastritis gastric cancer and chronic cancer samples were 10.40%, 15.70%, 96.50%, 0.60% and 3.14%, respectively. *H. pylori* strains harbored the highest prevalence of resistance against ampicillin (74.4%), clarithromycin (63.4%), trimethoprim (61.5%) and metronidazole (61.5%). The most commonly detected genotypes among the *H. pylori* strains isolated from different types of biopsy samples were *cagA* (84.79%), *vacA m2* (55.95%), *vacA s1a* (49.84%), *cagE* (48.58%), *iceA1* (47.02%) and *iceA2* (47.02%).

Conclusion: High prevalence of antibiotic resistance and virulent genotypes indicates an important public health issue. Similarities in antibiotic resistance and genotyping pattern of *H. pylori* strains isolated from humans and animals may show their similar routes of infection.

Keywords: *Helicobacter pylori*, virulence factor, antibiotic resistance, genotypes, clinical samples

Introduction

Helicobacter pylori (*H. pylori*) is a microaerophilic and Gram-negative spiral coccoid flagellated bacterium with 2–4 µm length and 0.5–1 µm width. It is known as one of the main causative agents of duodenal ulcer, peptic ulcer disease, gastric adenocarcinoma, type B gastritis and gastric B-cell lymphoma.^{1–3} Human stomach is considered as a main reservoir of *H. pylori* strains.^{1,2,4} In keeping with this, animals such as cows, sheep and goats may play an imperative role in the transmission of *H. pylori* infections to humans.^{5–7} The prevalence of *H. pylori* infections in some countries such as Iran is very high, and epidemiological studies showed that more than 50% of the human population are affected with *H. pylori* strains.^{8,9}

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H. pylori bacteria colonize the superficial parts of the gastric mucosa.^{8,10,11} It acts as an invasive bacterium and causes several gastrointestinal disorders and lesions in the gastric mucosa. Different pathogenic pathways have been developed for *H. pylori* infection. Signaling pathways were shown to be perturbed in gastric epithelial cells by virulence factors of *H. pylori* bacteria.¹²⁻¹⁴ Some of the most important virulence genes of this bacterium are the outer inflammatory protein (*oip*), vacuolating cytotoxin A (*vacA*), induced by contact with the epithelium antigen (*iceA*), blood group antigen-binding adhesion (*babA*) and cag PAI (cag pathogenicity island).¹⁵ Colonization, adhesion and invasion of *H. pylori* strains in gastric epithelial cells are facilitated by the presence of different virulence genes.^{16,17} *vacA* and cytotoxin associated gene A (*cagA*) are two important virulence genes with high importance in the pathogenicity of *H. pylori* infections.^{18,19} The *vacA* gene is polymorphic, comprising variable signal regions (type *s1* or *s2*) and mid-regions (type *m1* or *m2*). The *s1* type is additionally divided into *s1a*, *s1b* and *s1c* and the *m1* into *m1a* and *m1b* subtypes. The *cagA* gene has been detected in the severe cases of gastrointestinal disorders and peptic ulcers.^{16,17} The *iceA* gene has two main allelic variants: *iceA1* and *iceA2*. A small percentage of the *H. pylori* genome is predicted to encode outer membrane proteins (OMPs). The *oipA* gene plays an important role in efficient colonization of *H. pylori* into the mucosa.²⁰ Genotyping using these virulence markers is considered as one of the best approaches to study the correlations between *H. pylori* isolates from different samples.⁷

Antibiotic therapy is one of the best aspects of treatments for *H. pylori* infections. However, therapeutic options have become somewhat restricted because of the presence of severe resistance in some strains of this bacterium.⁶ Documented data disclosed that *H. pylori* strains isolated from clinical infections harbored high prevalence of resistance against different types of antibiotics including fluoroquinolone, tetracyclines, penicillins, aminoglycosides, sulfonamides and macrolides.⁷ Therefore, it is important to know the exact antibiotic resistance pattern of *H. pylori* strains isolated from human and animal clinical infections.

Data on the epidemiology and transmission of *H. pylori* is extremely significant in order to prevent its distribution and to identify high-risk populations, especially in areas that have high rates of infections such as Iran. Considering the indistinct epidemiological aspects of *H. pylori* in human and animal clinical samples and the high prevalence of *H. pylori* all-around the world, the present investigation was done in order to study the prevalence rate, genotyping patterns and

antibiotic resistance pattern of *H. pylori* strains isolated from human and animal clinical samples.

Methods

Sample collection

Number of samples to be collected was obtained based on the prevalence data of *H. pylori* found in recent studies and using the following equation:

$$N = \frac{(Z)^2 \times pq}{d^2}$$

whereas *p* is the mean prevalence of *H. pylori* in recent studies, (*Z*)² is the abscissa of the standard curve that cuts off an area α at the tails, *d* is the acceptable sampling error and *N* is the sample size.

The present cross-sectional study was conducted in the period between December 2016 and March 2017. Samples were collected from different parts of the Chaharmahal va Bakhtiari Province, Iran. Chaharmahal va Bakhtiari Province is located in an area of 16,411 km² between 31 degrees and 9 minutes to 32 degrees and 48 minutes of north latitude and 49 degrees and 28 minutes to 51 degrees and 25 minutes of east longitude from Greenwich meridian (southwest of Iran).

Samples were randomly collected from the gastric biopsies of humans (children [≤ 14 years] [*n*=50] and adults [650 samples: 408 females and 242 males] and animals (450 samples: 150 cows, 150 sheep and 150 goats). All patients were referred to the Hajar Hospital for upper gastrointestinal endoscopy. Patients with a history of partial gastric resection or previous treatment for *H. pylori* infection were excluded. Gastric biopsy samples of antrum were taken from humans. Samples of animals were taken from slaughterhouses in the same region as the humans. Samples from the rumen of animals in size of 2×4×4 mm³ were obtained immediately after slaughtering. All samples were kept in transport medium consisting of thioglycolate with 1.3 g/L agar with 2% yeast extract and transmitted to the Biotechnology Research Center of the Islamic Azad University of Shahrekord Branch in cooler with ice packs.

Isolation of *H. pylori*

Three different methods were used for diagnosis of *H. pylori* strains in the studied samples. All samples were cultured on Brucella Agar (EMD Millipore, Billerica, MA, USA) containing 5 mg/L of campylobacter selective supplement (EMD Millipore), 30 mg/L of colistin methanesulfonate, 100 mg/L of cycloheximide, 30 mg/L of nalidixic acid, 30 mg/L of trimethoprim, 10 mg/L of vancomycin (Sigma-Aldrich Co., St

Louis, MO, USA), amphotericin B, 7% sheep blood and 6% fetal calf serum (EMD Millipore). Media were then incubated at 37°C for 3–10 days on microaerophilic condition (85% N₂, 10% CO₂ and 5% O₂). Grown bacteria were identified using colony morphology, urease activities and positive reactions to oxidase, catalase and Gram staining. For histology method, the specimens were fixed in 10% buffered formalin and then embedded in paraffin and 5 mm thick sections were cut. Serial sections (biopsies ranged from 2×4×4 mm³) were stained with hematoxylin–eosin and May–Giemsa for histologic examination. Using endoscopy assay, gastric cancer and peptic ulcer were identified, and also gastritis in the peptic ulcer or gastric malignancy were diagnosed by an expert professor.

DNA extraction and genotyping of *H. pylori* strains

Typical colonies were subcultured on Wilkins-Chalgren anaerobe broth (EMD Millipore) supplemented with and cultured in similar conditions mentioned above. All tubes were incubated for 7 days at 37°C with shaking under microaerophilic conditions. Then, genomic DNA was extracted from grown colonies using a DNA extraction kit for cells and tissues (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. After extraction, the DNA samples were quantified (NanoDrop, Thermo Fisher Scientific), their purity checked (A260/A280) and their concentrations adjusted to 50 ng/μL. The integrity of the DNA was evaluated on a 2% agarose gel stained with ethidium bromide (0.5 μg/mL) (Thermo Fisher Scientific). The DNA concentration was also estimated by spectrophotometric absorbance at 257 nm (DR5000, Hach Company, Loveland, CO, USA).²¹ The DNA was stored at –20°C pending subsequent PCR analysis.

Extracted DNA was amplified for the *16S rRNA* gene (primers: HP-F: 5'-CTGGAGAGACTAAGCCCTCC-3' and HP-R: 5'-ATTACTGACGCTGATTGTGC-3').⁶ The genotypes of *vacA* (*s1a*, *s1b*, *s1c*, *m1a*, *m1b* and *m2*) and the presence of *iceA1*, *iceA2*, *oipA*, *cagA*, *cagE* and *babA2* genotypes were determined using PCR technique. The primers used in this study are listed in Table 1. All PCR mixtures were prepared in a final volume of 25 μL containing 50 ng DNA from the samples served as the template for PCR performed in a thermal cycler (Mastercycler gradient, Eppendorf, Germany), 1 μM of each primer, 2 mM MgCl₂, 5 μL of 10× PCR buffer, 200 μM dNTPs and 1 unit of Taq DNA polymerase (CinnaGen Co, Tehran, Iran). Thermal amplification of PCR products was performed at 95°C for 5 minutes and then for 32 cycles of 94°C for 1 minute, 61°C for 40 seconds, 72°C for 40 seconds and a final

extension at 72°C for 5 minutes, with a final hold at 10°C in a PCR thermal cycler (Mastercycler gradient). The PCR amplified products (10 μL) were subjected to electrophoresis in 1.5% agarose gel in 1× TBE buffer at 80 V for 30 minutes stained with a solution of ethidium bromide (EMD Millipore), and examined under Ultra Violet illumination (Cleaver Scientific Ltd, Rugby, UK).

Antimicrobial resistance pattern

The pure cultures of *H. pylori* strains were employed for antibiotic susceptibility test. One strain from each *H. pylori*-positive sample was selected and then subjected to the Kirby-Bauer disc diffusion method using Mueller-Hinton agar (EMD Millipore) supplemented with 5% defibrinated sheep blood and 7% fetal calf serum, according to the Clinical Laboratory Standards Institute (CLSI 2015).²² The antimicrobial resistance pattern of *H. pylori* was measured against the widely used antibiotics in cases of *H. pylori* gastric ulcer. The following antimicrobial disks (Thermo Fisher Scientific) were used: metronidazole (5 μg), ampicillin (10 μg), erythromycin (5 μg), clarithromycin (2 μg), furazolidone (1 μg), levofloxacin (5 μg), amoxicillin (10 μg), trimethoprim (25 μg), moxifloxacin (5 μg), cefsulodin (30 μg), tinidazole (5 μg) and ciprofloxacin (5 μg) were used. After incubation at 37°C for 48 hours in a microaerophilic atmosphere (5% oxygen, 85% nitrogen and 10% CO₂) using MART system (Anoxomat, Lichtenvoorde, the Netherlands), the susceptibility of the strains was measured against each antimicrobial agent. Results were construed in accordance with interpretive criteria provided by CLSI (2015). *H. pylori* ATCC 43504 was used as quality control organism in the antimicrobial susceptibility determination. The results were interpreted as resistant (R).

Statistical analyses

Data were transferred to Microsoft Excel spreadsheet (version 15; Microsoft Corporation, Redmond, WA, USA) for analysis. Using statistical software (version 16; SPSS Inc., Chicago, IL, USA), chi-squared test and Fisher's exact two-tailed test analysis were performed and the differences were considered significant at values of $P < 0.05$. Distribution of *H. pylori* genotypes isolated from gastric biopsy samples and also their antibiotic resistance pattern were statistically analyzed.

Ethical approval

The study was approved by the Ethical Council of Research of the Molecular Biology Research Center, Systems Biology and Poisonings Institute, Baqiyatallah University of Medical

Table 1 PCR primers and PCR conditions used for genotypes of *H. pylori* strains in this study

Genes*	Primer sequence (5'-3')*	Size of product (bp)*	Annealing
<i>s1a</i>	F: CTCTCGCTTTAGTAGGAGC R: CTGCTTGAATGCGCCAAAC	213	64
<i>s1b</i>	F: AGCGCCATACCGCAAGAG R: CTGCTTGAATGCGCCAAAC	187	64
<i>s1c</i>	F: CTCTCGCTTTAGTGGGGYT R: CTGCTTGAATGCGCCAAAC	213	64
<i>s2</i>	F: GCTAACACGCCAAATGATCC R: CTGCTTGAATGCGCCAAAC	199	64
<i>m1a</i>	F: GGTCAAAATGCGGTCATGG R: CCATTGGTACCTGTAGAAAC	290	65
<i>m1b</i>	F: GGCCCCAATGCAGTCATGGA R: GCTGTTAGTGCCTAAAGAAGCAT	291	65
<i>m2</i>	F: GGAGCCCCAGGAAACATTG R: CATAACTAGCGCCTTGCA	352	62
<i>cagA</i>	F: GATAACAGCCAAGCTTTTGAGG R: CTGCAAAAGATTGTTTGGCAGA	300	56
<i>iceA1</i>	F: GTGTTTTTAACCAAAGTATC R: CTATAGCCASTYTCTTTGCA	247	56
<i>iceA2</i>	F: GTTGGGTATATCACAATTTAT R: TTRCCCTATTTCTAGTAGGT	299/334	53
<i>oipA</i>	F: GTTTTTGATGCATGGGATTT R: GTGCATCTCTTATGGCTTT	401	55
<i>cagE</i>	F: TTGAAAACCTCAAGGATAGGATAGAGC R: GCCTAGCGTAATATCACCATTACCC	508	54
<i>BabA2</i>	F: CCAAACGAAACAAAAGCGT R: GCTTGTGTAAGCCGTCGT	271	55

Notes: *Table adapted from Ranjbar R, Khamesipour F, Jonaidi-Jafari N, Rahimi E. *Helicobacter pylori* in bottled mineral water: genotyping and antimicrobial resistance properties. *BMC Microbiol.* 2016;16:1–10. © Ranjbar et al 2016.⁴⁶

Sciences, Tehran, Iran (Consent Ref. Number BMSU-2017) (this study was conducted in accordance with the Declaration of Helsinki). Verification of this research project and the licenses related to the sampling process were approved by the Prof Reza Ranjbar (Approval Ref. Number 2017/33). Samples were collected from volunteer patients who were referred to the hospital for routine checkup. Written informed consent was obtained from all patients or their parents. Name, surname and other personal information of patients of the present study were kept secret. Additionally, we confirm that all methods were performed in accordance with the relevant guidelines and regulations.

Results

Clinical isolates

Totally, 112 ruminants, 19 children and 507 adult human gastric biopsy samples were studied for the presence of *H. pylori*. No relationship was found between the prevalence of *H. pylori* infection and the age of patients ranging from 3 to 72 years. Figure 1 represents the prevalence of *H. pylori* strains isolated from different types of gastric biopsy samples. We found that the prevalence of *H. pylori* strains in gastric biopsy samples studied using the urease test, culture and histological examination was 57.04%, 55.40% and 60.80%,

respectively. Based on the culture method, samples which were collected from adult humans (78%) and sheep (70%) had the highest prevalence of *H. pylori* strains, while those of goats (0.6%) and cows (4%) had the lowest. There were no statistically significant differences between three different diagnostic methods. Findings of the culture method were confirmed using the PCR-based amplification of *16S rRNA*. Table 2 represents the prevalence of *H. pylori* strains among different pathologic lesions of studied human. Distribution of gastric ulcers, duodenal ulcers and chronic gastritis among 700 human gastric biopsy samples were 10.40%, 15.70% and 96.50%, respectively. Distribution of gastric cancer and chronic cancer were 0.60% and 3.14%, respectively.

Antimicrobial resistance pattern

Table 3 represents the antibiotic resistance pattern of *H. pylori* strains isolated from different types of gastric biopsy samples. We found that *H. pylori* strains harbored the highest prevalence of resistance against ampicillin (74.4%), clarithromycin (63.4%), trimethoprim (61.5%), metronidazole (61.5%), erythromycin (60.1%) and amoxicillin (60.1%). Prevalence of resistance against cefsulodin, furazolidone and moxifloxacin was low. Statistically significant differences

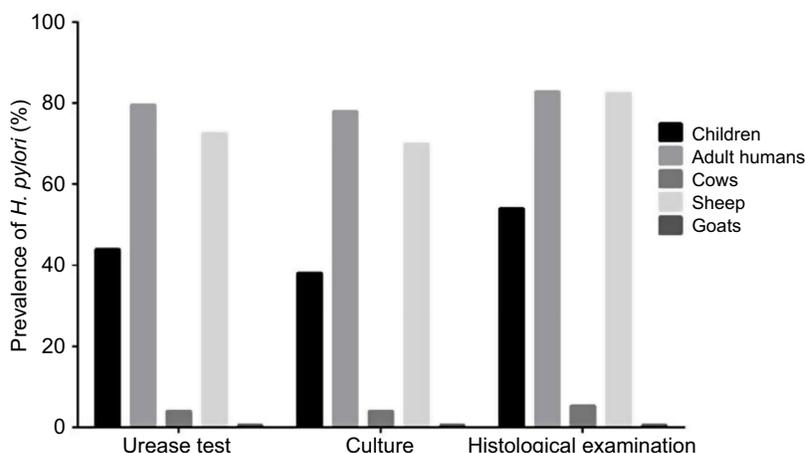


Figure 1 Prevalence of *H. pylori* infection determined by diagnostic tests.

Table 2 Prevalence of *H. pylori* infection in patients (adult humans and children)

Type of samples (no. of positive)	Prevalence of <i>H. pylori</i> (%)				
	Gastric ulcers	Duodenal ulcers	Gastric cancer	Chronic gastritis	Chronic cancer
Adult humans (507)	71 (10.1)	107 (15.2)	4 (0.62)	659 (94.1)	16 (2.2)
Children (19)	2 (10.5)	3 (15.7)	0 (0.0)	17 (89.4)	6 (31.5)

were found between the type of samples and prevalence of antibiotic resistance ($P < 0.05$).

Genotyping pattern

Table 4 represents the distribution of different genotypes among the *H. pylori* strains isolated from different types of gastric biopsy samples. Total distribution of *cagE*, *cagA*, *iceA1*, *iceA2*, *oipA* and *babA2* virulence factors was 48.5%, 87.3%, 47%, 47%, 15.2% and 27.4%, respectively. We found that the most commonly detected genotypes among the *H. pylori* strains isolated from different types of biopsy samples were *cagA* (84.79%), *vacA m2* (55.95%), *vacA s1a* (49.84%), *cagE* (48.58%), *iceA1* (47.02%) and *iceA2* (47.02%). Prevalence of *vacA mib* (6.58%), *oipA* (15.20%) and *vacA s2* (14.42%) was low. Statistically significant differences were found between the type of samples and prevalence of different genotypes ($P < 0.05$).

Discussion

H. pylori is an opportunistic and infectious bacterium in both humans and animals. It is also a causative agent of gastritis, duodenal ulcers, gastric ulcer and cancer in both humans and animals.^{23,24} However, studies conducted rarely in this field on animal cases,⁵⁻⁷ and all of them have demonstrated the role of the *H. pylori* strains as a causative agent of gastrointestinal diseases in animals.²³⁻²⁶ High prevalence of *H. pylori* strains

in different types of foods with animal origins showed that animals and especially ruminants may play an important role in the maintenance and transmission of infection to humans.

As far as we know, the present study is one of the most comprehensive reports of prevalence and genotype of the *H. pylori* strains isolated from both humans and animals. Our findings showed that the prevalence of *H. pylori* strains in gastric biopsy samples of cows, sheep and goats was 4%, 70% and 0.66%, respectively. Prevalence of *H. pylori* strains in gastric ulcer, duodenal ulcer, gastric cancer, chronic gastritis and chronic cancer of adults and children was 10.1% and 10.5%, 15.2% and 15.7%, 0.62% and 0%, 94.1% and 89.4% and finally 2.28% and 31.5%, respectively. High prevalence of *H. pylori* in our study maybe due to the fact that Iran is one of the main sites of *H. pylori* infections in the world and therefore, pathogenic bacteria can easily transmit from infected animals or humans to healthy ones. High prevalence of *H. pylori* infection has also been reported in Iran.^{27,28} Another important reason is the fact that all samples were taken from the sites of *H. pylori* infection and the distribution of *H. pylori* strains is higher than other bacteria that exist in the site of the gastric ulcer, duodenal ulcer, gastric cancer, chronic gastritis and chronic cancer in both humans and animals.^{26,29-31} There were also many studies that showed the higher prevalence of *H. pylori* in the sites of gastrointestinal disorders in both humans and animals.^{32,33} Another important reason maybe the high prevalence of *H. pylori* strains in several types

Table 3 Antibiotic resistance profile of *H. pylori* isolates of samples

Type of samples (no. of positive)	Antibiotic resistance pattern (%)												
	AM10	Met5	ER5	CIR2	AMX10	Cet30	TRP25	FZLI	MOX5	TIN4	CIP5		
Cows (6)	5 (83.3)	4 (66.6)	4 (66.6)	4 (66.6)	4 (66.6)	1 (16.6)	4 (66.6)	2 (33.3)	1 (16.6)	3 (50.0)	3 (50.0)		
Sheep (105)	72 (68.5)	62 (59)	58 (55.2)	65 (61.9)	48 (45.7)	27 (25.7)	64 (60.9)	37 (35.2)	22 (20.9)	42 (40.0)	51 (48.5)		
Goats (1)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	0 (0.0)	1 (100)	0 (0.0)	0 (0.0)	1 (100)	1 (100)		
Adults (507)	380 (74.9)	314 (61.9)	308 (60.7)	321 (63.3)	318 (62.7)	127 (25.0)	315 (62.1)	137 (27.0)	121 (23.8)	252 (49.7)	248 (48.9)		
Children (19)	17 (89.4)	12 (63.1)	13 (68.4)	14 (73.6)	13 (68.4)	2 (10.5)	11 (57.8)	4 (21.0)	2 (10.5)	9 (47.3)	9 (47.3)		
Total (638)	475 (74.4)	393 (61.5)	384 (60.1)	405 (63.4)	384 (60.1)	157 (24.6)	395 (61.9)	180 (28.2)	146 (22.8)	307 (48.1)	312 (48.9)		

Abbreviations: AM10, ampicillin (10 µg); Met5, metronidazole (5 µg); ER5, erythromycin (5 µg); CLR2, clarithromycin (2 µg); AMX10, amoxicillin (10 µg); Lev5, levofloxacin (5 µg); Ceft30, cefsulodin (30 µg); TRP25, trimethoprim (25 µg); FZLI, furazolidone (1 µg); Mox5, moxifloxacin (5 µg); Tin, tinidazole (prepared from pure powders, Sigma-Aldrich Co, 5 µg); CIP5, ciprofloxacin (5 µg).

of food samples reported in previous Iranian researches.^{5,6,34,35} Therefore, it is not surprising that the *H. pylori* strains can easily transmit from the foods to humans and animals.

The prevalence of *H. pylori* in our study was around 40%–80% which is similar to previous reports from Iran, South America, Japan, Turkey and Pakistan, where more than 80% of dyspepsia patients were *H. pylori* positive; however, in Scandinavia and England, the prevalence ranges between 20% and 40%.^{36,37} The diversity of *H. pylori* frequency in numerous hosts and regions may relate to animal, microbe and environmental factors.

Another part of our investigation focused on the prevalence of antibiotic resistance in the *H. pylori* strains isolated from animals and humans. We found that bacterial strains harbored a high prevalence of resistance against commonly used antibiotics in the field of *H. pylori* infections. Prescription of ampicillin, amoxicillin, metronidazole, erythromycin and clarithromycin is common in the treatment of cases of *H. pylori* infections in Iran. Therefore, it is not surprising that the majority of bacterial strains were resistant against these types of antibiotics. Unauthorized and irregular prescription of antibiotics and also self-treatment of infections by patients are the main factors that affected the high prevalence of antibiotic resistance. Another Iranian study³⁸ reported the high prevalence of resistance of *H. pylori* strains against ampicillin, amoxicillin, metronidazole, erythromycin and clarithromycin. Recent meta-analysis study³⁹ showed that the rates of *H. pylori* antibiotic resistance reported from Asian, American, African and European countries were 47.22% (30.5%–75.02%) for metronidazole, 19.74% (5.46%–30.8%) for clarithromycin, 18.94% (14.19%–25.28%) for levofloxacin, 14.67% (2%–40.87%) for amoxicillin, 11.70% (0%–50%) for tetracycline, 11.5% (0%–23%) for furazolidone and 6.75% (1%–12.45%) for rifabutin. Because clarithromycin is the most potent antibiotic involved in the management of *H. pylori* infections, resistance to clarithromycin is important. We found that *H. pylori* strains showed high prevalence of resistance against clarithromycin (61%–100%). This prevalence of resistance against clarithromycin was higher than those in Norway (5.9%), Spain (32.01%) and Portugal (42.35%).³⁹ Besides, the prevalence rate was also higher than India (58.8%), China (46.54%) and Malaysia (2.4%).³⁹ This finding is due to the higher prescription of the macrolides in Iran. In recent years, due to extensive prescription of clarithromycin especially for the infections of the respiratory tracts especially in children, clarithromycin resistance has increased in various regions.^{38,39} Metronidazole is used to treat *H. pylori* infections and is one of the few antibacterial agents as a drug of choice that

Table 4 Total distribution of various genotypes in *H. pylori* strains of positive samples

Type of samples (no. of positive)	Antibiotic resistance pattern (%)												
	CagE	CagA	IceA1	IceA2	OipA	BabA2	VacA s1a	VacA s1b	VacA s1c	VacA s2	VacA m1a	VacA m1b	VacA m2
Cows (6)	3 (50.0)	5 (83.3)	3 (50)	3 (50.0)	1 (16.6)	2 (33.3)	6 (100)	2 (33.3)	0 (0.0)	2 (33.3)	2 (33.3)	0 (0.0)	5 (83.3)
Sheep (105)	54 (51.4)	88 (83.8)	48 (45.7)	48 (45.7)	11 (10.4)	17 (16.1)	51 (48.5)	39 (37.1)	0 (0.0)	14 (13.3)	46 (43.8)	0 (0.0)	22 (20.9)
Goats (1)	1 (100)	1 (100)	1 (100)	1 (100)	0 (0.0)	0 (0.0)	1 (100)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100)	0 (0.0)	1 (100)
Adults (507)	243 (47.9)	446 (87.9)	238 (46.9)	238 (46.9)	82 (16.1)	152 (29.9)	251 (49.5)	68 (13.4)	106 (20.9)	74 (14.5)	168 (33.1)	42 (8.2)	312 (61.5)
Children (19)	9 (47.3)	17 (89.4)	10 (52.6)	10 (52.6)	3 (15.7)	4 (21.0)	9 (47.3)	3 (15.7)	2 (10.5)	2 (10.5)	2 (10.5)	0 (0.0)	17 (17.0)
Total (638)	310 (48.5)	541 (84.7)	300 (47.0)	300 (47.0)	97 (15.2)	175 (27.4)	318 (49.8)	112 (17.5)	108 (16.9)	92 (14.4)	219 (34.3)	42 (6.5)	357 (55.9)

is effective in eradicating the *H. pylori*. In keeping with this, this antibiotic is used for the treatment of transient gastrointestinal and anaerobic infections in Iran. The rate of self-prescription and also self-treatment with this antibiotic is high among the Iranian patients. It is also used for the treatment of gynecological, dental and parasitic-related infectious diseases. Therefore, it is not surprising that majority of *H. pylori* strains were resistant against this antibiotic agent (59%–100%). Literature-based reports showed that metronidazole resistance is the most common antibiotic resistance in *H. pylori* and overall metronidazole resistance found in 47.22% in descending order in Africa 75.02%, South America 52.85%, Asia 46.57%, Europe 31.19%, to 30.5% in North America.³⁹ Amoxicillin is another important choice drug for treatment of *H. pylori* infections in Iran. It is mainly used for the treatment of *H. pylori* infections in regions with high prevalence of resistance against metronidazole. We found that the prevalence of resistance against amoxicillin was lower than other tested antibiotics (10%–24%). The prevalence of amoxicillin resistance in American and European countries is low from zero in Norway, Germany, Finland and Poland, 1.4% in Spain to 2% in the United States.¹⁶ However, the prevalence of resistance against this antibiotic in South Nigeria, Colombia, India and Africa was 66%, 20.5%, 72.5% and 97.5%, respectively.³⁹

Another part of our study focused on the detection of putative genotypes of the *H. pylori* strains. We found that *H. pylori* strains isolated from human clinical samples harbored the higher prevalence of *vacA*, *cagA*, *iceA*, *oipA* and *babA* genotypes. However, the prevalence of these genotypes among the animal-based strains was also high. Prevalence of *cagE* genotype in *H. pylori* strains recovered from animals was higher than humans. *H. pylori* strains isolated from the biopsy samples of goats harbored the highest prevalence of studied genotypes. Similar profile of the genotypes among the *H. pylori* strains isolated from humans and animals maybe due to the zoonotic aspect of the bacteria. There were several studies that reported the high prevalence of isolation of *H. pylori* from foods with animal origins.^{5,6,35,40,41} Therefore, it is not far from the mind that the *H. pylori* strains isolated from humans and animals of our study have common primary origins or have been transmitted from animals to humans or vice versa. Detection of mentioned genotypes in animals shows that the *H. pylori* strains may have significant roles in occurrence of gastrointestinal diseases and disorders in cows, sheep and goats.

There was only one research about the comparison of the prevalence and genotype pattern of the *H. pylori* strains between humans and animals.²⁶ This study showed that

overall 6 (3%) cows, 32 (16%) sheep and 164 (82%) human being specimens were positive for *H. pylori* strains. The *vacA s1a/m1a* was the predominant *H. pylori* genotype in cow, sheep and human samples. There was 3.4%–8.4% variability and 92.9%–98.5% homology between the genotypes of the *H. pylori* strains isolated from sheep and human samples. Another study⁴² that was conducted on food samples reported that the most commonly detected genotypes were *vacA s1a* (78.37%), *vacA m2*(75.67%), *vacA m1a* (51.35%) and *cagA* (41.89%). They showed that the prevalence of *iceA1*, *iceA2* and *oipA* genotypes was 13.51%, 4.05% and 18.91%, respectively, which was lower than our findings.

The geographic distribution of different *H. pylori* genotypes remains mainly unknown. Prevalence of virulent genotypes in Europe and America may have significant epidemiological consequences that are linked to the presence of the *cagA* gene and the severity of *H. pylori*-related diseases.⁴³ *CagA* is the most extensively studied *H. pylori* virulence factor. Prevalence of *cagA*-positive strains in Europe is around 60%–70%, while the prevalence in Asian countries is 90%.⁴³ Such differences in the prevalence of *cagA* genotypes could not be clarified exactly; however, they have been related to the genetic heterogeneity or to differences in the geographic locations and maybe differences in the food habits. Documented data reported that *cagA* genotypes are the main factors responsible for the occurrence of gastric cancer and other malignant neoplasms in animal models.²⁰ Since it has been demonstrated that *H. pylori* carries only a single copy of the *vacA* gene, detection of multiple genotypes implies the presence of multiple strains in a clinical sample. We found that the *vacA s1a* and *m2* were predominant genotypes in the *H. pylori* strains. These genotypes are accompanied by the occurrence of human clinical and gastrointestinal diseases.^{44,45} *VacA* is the second most widely studied *H. pylori* virulence factor. It can induce multiple cellular activities, including membrane channel formation, cytochrome c release from mitochondria leading to apoptosis and binding to cell-membrane receptors followed by initiation of a proinflammatory response.²⁰

Conclusion

Total prevalence of *H. pylori* infection in the Chaharmahal va Bakhtiari Province, Iran, was high. Virulence factors (*vacA*, *cagA*, *oipA*, *cagE*, *BabA2* and *iceA*) of *H. pylori* in Iran might contribute to the low rate of gastric cancer. However, the presence of *cagA* is associated with more severe clinical outcomes of gastroduodenal diseases. High prevalence of virulent and resistant *H. pylori* strains shows a significant public health problem. Similarity in the antibiotic resistance pattern

and also distribution of different genotypes in the *H. pylori* strains of human and animal sources may show that they had similar routes of infections. Additionally, similar antibiotic resistance and genotyping pattern may show that animals and especially ruminants are the probable sources for transmission of *H. pylori* infections to human population. However, additional studies are required to determine the exact epidemiological aspects of *H. pylori* infections in humans and animals.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Disclosure

The authors report no conflicts of interest in this work.

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