Virulence factors and antibiotic resistance of *Helicobacter pylori* isolated from raw milk and unpasteurized dairy products in Iran

Soolmaz Mousavi¹, Farhad Safarpour Dehkordi¹ and Ebrahim Rahimi²*

**Abstract**

**Background:** Despite the high importance of *Helicobacter pylori*, the origin and transmission of this bacterium has not been clearly determined. According to controversial theories and results of previous studies, animal source foods – especially milk – play an important role in the transmission of *H. pylori* to humans. The aim of the present study was to determine the distribution of *vacA*, *cagA*, *iceA* and *oipA* virulence factors in *H. pylori* strains isolated from milk and dairy products and study their antimicrobial resistance properties.

**Methods:** A total of 520 raw milk and 400 traditional dairy product samples were cultured and tested. Those that were *H. pylori*-positive were analyzed for the presence of *vacA*, *cagA*, *iceA* and *oipA* virulence factors. Antimicrobial susceptibility testing was performed by the disk diffusion method.

**Results:** One hundred and three out of 520 milk samples (19.8%) and 77 out of 400 dairy product samples (19.2%) were contaminated with *H. pylori*. The most frequently contaminated samples were ovine milk (35%) and traditional cheese (30%). Total prevalence of *vacA*, *cagA*, *iceA* and *oipA* factors were 75%, 76.6%, 41.6% and 25%, respectively. *H. pylori* strains of milk and dairy products harbored high levels of resistance to ampicillin (84.4%), tetracycline (76.6%), erythromycin (70.5%) and metronidazole (70%).

**Conclusions:** High presence of antibiotic-resistant strains of *H. pylori* suggest that milk and dairy samples may be the sources of bacteria that can cause severe infection. Our findings should raise awareness about antibiotic resistance in *H. pylori* strains in Iran.

**Keywords:** *Helicobacter pylori*, Virulence factors, Antibiotic resistance properties, Milk, Dairy products, Iran

**Background**

Milk plays an important role in the nutrition of Iranian people since it is considered a complete food source, particularly for children and the elderly. Milk of animal origin is usually manufactured into more stable dairy products of worldwide commerce such as yogurt, cream, butter and cheese. Every day millions of people consume milk and dairy products. Therefore, hygienic quality of produced milk is extremely important regarding public health hazards.

*Helicobacter pylori* is a microaerophilic gram-negative bacterium with a curved spiral shape which is known as the causative agent of type B gastritis, peptic ulcer disease, gastric adenocarcinoma and mucosa-associated lymphoid tissue lymphoma [1]. In spite of the general idea about the low prevalence of gastric cancer, it is considered the fourth most common type of cancer and the second leading cause of cancer-related deaths worldwide [2]. A total of 1,665,540 new cancer cases and 585,720 cancer deaths are estimated to occur in the United States in 2014 [3].

Several studies have indicated the presence of *H. pylori* in the stomach of domestic animals in the absence of gastritis [4-7]. It was also isolated in milk of sheep, goat, cow, buffalo and camel species. These findings indicate that domestic ruminants may be a natural host for *H. pylori*. Moreover, the detection of *H. pylori* in the milk and feces of domestic animals suggests that *H. pylori* may be regarded as a zoonotic infection [4-7].

* Correspondence: Ebrahimrahimi55@yahoo.com
  ²Department of Food Hygiene, College of Veterinary Medicine, Islamic Azad University, Shahrekord Branch, PO Box 166, Shahrekord, Iran
  Full list of author information is available at the end of the article

© 2014 Mousavi et al; licensee BioMed Central Ltd. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.
Good conditions for the survival of *H. pylori* in animal milk provides opportunities for its transmission to humans [8]. At temperatures below 30°C, *H. pylori* is capable of surviving in milk and water, fresh fruit and vegetables, fresh meat (including red meat, poultry and fish) and some dairy products [8].

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 gastritis, peptic ulcers, and gastric cancer such as Iran [5-7]. In addition to routes of transmission, treatment is a critical point in the epidemiology of *H. pylori* infection in humans, since therapeutic options have become somewhat limited because of the presence of multidrug resistant strains of this bacterium [9]. Moreover, to the best of our knowledge, we could not find any published data on the antibiotic resistance pattern of *H. pylori* strains isolated from animal source foods.

Another aspect of *H. pylori* epidemiology, connected to its pathogenicity, is related to putative virulence factors. In fact, to determine the pathogenicity of *H. pylori*, assessment of latent virulence factors is required. Survival and success of pathogens require that they colonize the host, reach an appropriate niche, avoid host defenses, replicate, and exit the infected host to spread to an uninfected one. In *H. pylori*, the genes that are mostly associated with infections include cytotoxin-associated gene A (*cagA*), vacuolating cytotoxin (*vacA*), outer inflammatory protein (*oipA*) and finally the gene induced by contact with epithelium (*iceA*) [10,11]. These genes are usually related to adhesion to gastric epithelial cells [10,11].

It has been reported that the *cagA* gene is present in about 60% of *H. pylori* isolates from clinical samples. This gene is associated with inflammation by activation of NF-kB and secretion of cytokines and chemokines such as interleukin 8 (IL-8) [10,11]. The gene *vacA* plays a possible role on the formation anion-selective channels within artificial membranes and it is assumed to do the same in *vivo*, increasing absorbency to anions and urea [10,11]. Endocytosis of *vacA* channels leads to the formation of large vacuoles within the late endosome-lysosome cubicle [10,11]. The function of *iceA* gene is not yet clear. However, it is hypothesized that this gene is upregulated upon contact of *H. pylori* with the gastric epithelium and has been regarded as a marker for peptic ulcer diseases [12,13]. Finally, *oipA* induces IL-8 secretion by epithelial cells and increases inflammation as well as the clinically important presentation of peptic ulcer [12,13]. Considering the unclear epidemiological aspects of *H. pylori* infection, the present investigation was carried out in order to study the distribution of virulence factors and antibiotic resistance properties of *H. pylori* isolated from animal milk and dairy products.

**Methods**

**Samples**

Overall, 520 raw milk samples were collected from the following species: bovine (n = 120), caprine (n = 100), ovine (n = 100), buffalo (n = 80), camel (n = 60) and donkey (n = 60). The samples were obtained from farm bulk tanks and milk collection centers from several geographic regions of Iran, from March 2013 to March 2014. Bovine and buffalo samples were collected throughout this period. Because of the seasonality of their lactating periods, caprine, donkey and ovine milk samples were only available in certain months (from March through May and September to November in Iran). At each site, sampling was performed according to the International Dairy Federation guidelines [14]. Samples (100 mL, in sterile glass containers) were transported to the laboratory at 4°C within a maximum of 6 to 12 hours after collection.

For dairy products, 100 samples of cheese, 100 of butter, 100 of cream and 100 of ice cream made of raw milk were purchased from traditional supermarkets. All selected dairy products were made from unpasteurized milk and after collection were kept under refrigeration in plastic bags; information about date of production and shelf life were not available. These products are packed manually in traditional conditions and are popular among Iranian people because of their pleasant taste and smell. Dairy product samples were collected over a period of eight months (between August 2013 and February 2014), and were analyzed on the day of acquisition. Samples were transported under refrigeration (4-6°C) in thermal boxes containing ice packs and were tested immediately after collection.

**Isolation of Helicobacter pylori**

Twenty five milliliter or grams of each sample were added to 225 mL of Wilkins Chalgren anaerobe broth (Oxoid, UK) supplemented with 5% of horse serum (Sigma, USA) and colistin methanesulphonate (30 mg/L), cycloheximide (100 mg/L), nalidixic acid (30 mg/L), trimethoprim (30 mg/L), and vancomycin (10 mg/L) (Sigma, USA) and incubated for seven days at 37°C under microaerophilic conditions (86% N₂, 9% CO₂, 5% O₂) using the Bio-Bags (type CFi; Becton Dickinson). Then, 0.1 mL of the enrichment selective broth was plated onto Wilkins Chalgren anaerobe agar (Oxoid, UK) supplemented with 5% of defibrinated horse blood and 30 mg/L colistin methanesulphonate, 100 mg/L cycloheximide, 30 mg/L nalidixic acid, 30 mg/L trimethoprim, and 10 mg/L vancomycin (Sigma, USA) [15] and incubated for seven days at 37°C under microaerophilic conditions. Suspected colonies were identified as *H. pylori* based on the morphology of the colonies, Gram stain, and production of oxidase, catalase, and urease [16]. The isolates were identified as *H. pylori* by using conventional bacteriological methods, and
were also positive by the PCR assay. For comparison, a reference strain of *H. pylori* (ATCC 43504) was employed.

**Antimicrobial susceptibility testing**

Pure cultures of *H. pylori* isolates, one strain from each positive sample, were used for antimicrobial susceptibility testing. Tests were performed by the Kirby-Bauer disc diffusion method using Mueller-Hinton agar (HiMedia Laboratories, India) supplemented with 5% defibrinated sheep blood and 7% fetal calf serum, according to the Clinical and Laboratory Standards Institute guidelines (CLSI) [17]. The antimicrobial resistance of *H. pylori* was measured against the widely used antibiotics in cases of *H. pylori* gastric ulcer. The following antimicrobial impregnated disks (HiMedia Laboratories, India) were used: metronidazole (5 μg), ampicillin (10 μg), clarithromycin (2 μg), erythromycin (5 μg), tetracycline (30 μg), amoxicillin (10 μg), levofloxacin (5 μg), trimethoprim (25 μg), cef-sulodin (30 μg), and furazolidone (1 μg). After incubation at 37°C for 48 hours in a microaerophilic atmosphere, the susceptibility of the *H. pylori* to each antimicrobial agent was measured and the results were interpreted in accordance with criteria provided by CLSI [17]. *H. pylori* ATCC 43504 was used as quality control organism in antimicrobial susceptibility determination.

**DNA extraction and Helicobacter pylori 16S rRNA gene amplification**

Suspected colonies were identified as *H. pylori* though PCR technique. Genomic DNA was extracted from *H. pylori* colonies using a DNA extraction kit for cells and tissues (Roche Applied Science, Germany, 11814770001) according to the manufacturer’s instructions. Density of extracted DNA was assessed by optic densitometry. Extracted DNA was amplified for the 16S rRNA gene (primers: HP-F: 5'-CTGGAGAGACTAAGGCCCTCC-3' and HP-R: 5'-ATTACTGACGCTGTATGTCG-3') [18]. PCR reactions were performed in a final volume of 50 μL containing 5 μL 10 × buffer + MgCl₂, 2 mM Dntp (Fermentas, Germany), 2 unit Taq DNA polymerase (Fermentas, Germany), 100 ng genomic DNA as a template, and 25 picomole of each primer (Cinagen, Iran). PCR was performed using a thermal cycler (Flexcycler² Gradient, Eppendorf, Germany) under the following conditions: an initial denaturation of two minutes at 94°C; 30 cycles of 95°C for 30 seconds, 60°C for 30 seconds, 72°C for 30 seconds and a final extension at 72°C for eight minutes.

**Virulence factors of Helicobacter pylori**

The list of primers used for detection of vacA, cagA, oipA and iceA virulence factors in *H. pylori* is shown in Table 1. PCR was performed in a total volume of 50 μL that contained 1 μM of each primer, 1 μL of genomic DNA (approximately 200 ng), 1 mM dNTPs mix (Fermentas, Germany), 2 mM MgCl₂, and 0.05 of U/μL Taq DNA polymerase (Fermentas, Germany). PCR amplifications were performed in an automated thermal cycler (Mastercycler Gradient, Eppendorf, Germany). The following cycle conditions were used for PCR amplification: for vacA – 35 cycles of 30 seconds at 95°C, 60 seconds at 66°C, and five minutes at 72°C; for cagA – 35 cycles of 30 seconds at 95°C, 60 seconds at 55°C, and five minutes at 72°C; for iceA – 35 cycles of 30 seconds at 95°C, 60 seconds at 56°C, and five minutes at 72°C; and, finally, for oipA – 60 seconds at 94°C, 60 seconds at 52°C and 60 seconds at 72°C. All runs included one negative DNA control consisting of PCR grade water and two or more positive controls (26695, J99, SS1, Tx30, 88–183 and positive samples).

PCR products were resolved by agarose gel electrophoresis (5 V/60 min) using 1.5% agarose in Tris Acetate-EDTA (TAE) buffer containing 0.5 μg/mL of ethidium bromide (Merck, Germany). Molecular size ladder of 100 bp (Fermentas, Germany) was used to determine the size of the bands. The gel was viewed and photographed on a Gel-Doc System (Bio-Rad, USA). All tests were performed in triplicate.

**Statistical analysis**

Data were transferred to Microsoft Excel spreadsheet (Microsoft Corp., USA) for analysis. Using SPSS 16.0 statistical software (SPSS Inc., USA), chi-squared test and Fisher’s exact two-tailed test analysis were performed and differences were considered significant at values of *p* < 0.05. Distribution of virulence factors and antimicrobial resistance properties of *H. pylori* isolated from the milk and dairy products were statistically analyzed.

---

**Table 1** Oligonucleotide primers used for detection of vacA, cagA, oipA and iceA virulence factors of *Helicobacter pylori* strains isolated from milk and dairy products [19,20]

| Gene name | Primer sequence (5’-3’) | Size of product (bp) |
|-----------|------------------------|---------------------|
| vacA      | F: GCCGATATGCAATGAGCCGC | 678                 |
|           | R: CAATCGTGTGGGTTCTGGAGC |                    |
| cag A     | F: AATACACACAGGCCTCAAG | 400                 |
|           | R: TTGTTGCCGTTCCTCTC |                    |
| iceA      | F: CGTGCGTTAGCGTTAGAGATTT | 557               |
|           | R: TCATTGTATATCCATTTACAG |                  |
| oip A     | F: GTTTTTGATGCATGGGATT | 401                 |
|           | R: GTGATCTTCCTATGCGGTTT |                    |
Results and discussion
Prevalence of Helicobacter pylori in studied samples
Eight hundred and sixty milk and dairy samples were analyzed for the presence of H. pylori. Distribution of H. pylori in various types of milk and dairy products is shown in Table 2. Out of 920 milk and dairy samples, 180 (19.5%) were positive for H. pylori. Specifically, among the positive samples, 103 out of 520 were milk (19.8%) and 77 out of 400 consisted of dairy samples (19.2%). Ovine milk (35%) and traditional cheese (30%) were the most commonly contaminated products.

Table 2 Total distribution of Helicobacter pylori in various types of milk and dairy products

| Type of samples        | Number of samples | Occurrence of Helicobacter pylori (%) |
|------------------------|-------------------|---------------------------------------|
| Bovine raw milk        | 120               | 20 (16.6)                             |
| Ovine raw milk         | 100               | 35 (35)A*                             |
| Caprine raw milk       | 100               | 28 (28)                               |
| Buffalo raw milk       | 80                | 12 (15)a                              |
| Camel raw milk         | 60                | 8 (13.3)a                             |
| Donkey milk            | 60                | –                                     |
| Total milk             | 520               | 103 (19.8)                            |
| Traditional cheese     | 100               | 30 (30)b                              |
| Traditional cream      | 100               | 15 (15)                               |
| Traditional butter     | 100               | 5 (5)b                                |
| Traditional ice cream  | 100               | 27 (27)                               |
| Total dairy products   | 400               | 77 (19.2)                             |
| Total                  | 920               | 180 (19.5)                            |

The same small and capital letters in columns indicate a significant difference about p <0.05.

Distribution of virulence factors in Helicobacter pylori isolates
Distribution of putative virulence factors in H. pylori strains isolated from milk and dairy products is shown in Table 3. Of 180 isolated strains of H. pylori, the distribution of vacA, cagA, iceA and oipA virulence factors were 135 (75%), 138 (76.6%), 75 (41.6%) and 45 (25%), respectively. The most commonly detected virulence factor in H. pylori was cagA. Isolates from caprine milk and traditional cheese had the highest incidence of putative virulence genes.

Antimicrobial resistance pattern of Helicobacter pylori isolates
The susceptibility of H. pylori strains to ten commonly used commercial antimicrobial agents was studied in our investigation. Results showed that H. pylori strains harbored high levels of antibiotic resistance to ampicillin (84.4%), tetracycline (76.6%), erythromycin (70.5%) and metronidazole (70%) (Table 4). On the other hand, a lower frequency of resistance was observed against levofloxacin (12.7%), furazolidone (13.8%), clarithromycin (17.7%) and cefsulodin (21.1%).

To the best of our knowledge, the present study is the first investigation about the molecular detection of vacA, cagA, iceA and oipA virulence factors of H. pylori strains of bovine, ovine, caprine, buffalo and camel milk and their derived dairy products.

Total prevalence of H. pylori in bovine, ovine, caprine, buffalo and camel raw milk samples of our survey were 16.66%, 35%, 28%, 15% and 13.33%, respectively. Rahimi and Kheirabadi [5] reported that the incidence of H. pylori in raw bovine, ovine, caprine, buffalo and camel milk and their derived dairy products.

Table 3 Distribution of putative virulence factors in Helicobacter pylori strains isolated from various types of milk and dairy products

| Type of samples (n. positive) | vacA (n. positive) | cagA (n. positive) | iceA (n. positive) | oipA (n. positive) |
|-------------------------------|--------------------|--------------------|--------------------|--------------------|
| Bovine raw milk (20)          | 15 (75)            | 14 (70)            | 10 (50)            | 8 (40)             |
| Ovine raw milk (25)           | 22 (88)            | 23 (92)            | 12 (48)            | 5 (20)             |
| Caprine raw milk (28)         | 25 (89.2)          | 27 (96.4)          | 14 (50)            | 10 (35.7)          |
| Buffalo raw milk (12)         | 9 (75)             | 10 (83.3)          | 4 (33.3)           | 2 (16.6)           |
| Camel raw milk (8)            | 5 (62.5)           | 5 (62.5)           | 2 (25)             | 1 (12.5)           |
| Donkey raw milk (–)           | –                  | –                  | –                  | –                  |
| Total milk (103)              | 76 (73.7)          | 79 (76.6)A*        | 42 (40.7)          | 26 (25.2)b         |
| Traditional cheese (30)       | 26 (86.6)          | 26 (86.6)          | 15 (50)            | 8 (26.6)           |
| Traditional cream (15)        | 10 (66)            | 11 (73.3)          | 7 (46.6)           | 4 (26.6)           |
| Traditional butter (5)        | 3 (60)             | 3 (60)             | 1 (20)             | –                  |
| Traditional ice cream (27)    | 20 (74)            | 19 (70.5)          | 10 (37)            | 7 (25.9)           |
| Total dairy products (77)     | 59 (76.6)          | 59 (76.6)b         | 33 (42.8)b         | 19 (24.6)b         |
| Total (180)                   | 135 (75)           | 138 (76.6)c        | 75 (41.6)          | 45 (25)c           |

The same small and capital letters in rows indicate significant differences about p <0.05.
Table 4 Antibiotic resistance of *Helicobacter pylori* strains isolated from various types of milk and dairy products

| Type of samples (n. positive) | METR5 | AM10 | CLRT2 | ERT5 | TE30 | AMX10 | FZL1 | Lev5 | TRP25 | Cef30 |
|------------------------------|-------|------|-------|------|------|-------|------|------|-------|-------|
| Bovine raw milk (20)         | 15 (75) | 18 (90) | 4 (20) | 17 (85) | 17 (85) | 15 (75) | 3 (15) | 4 (20) | 8 (40) | 5 (25) |
| Ovine raw milk (25)          | 19 (76) | 22 (88) | 5 (20) | 19 (76) | 20 (80) | 16 (64) | 4 (16) | 5 (20) | 6 (24) | 6 (24) |
| Caprine raw milk (28)        | 21 (75) | 25 (89.2) | 6 (21.4) | 20 (71.4) | 22 (78.5) | 19 (67.8) | 4 (14.2) | 4 (14.2) | 10 (35.7) | 6 (21.4) |
| Buffalo raw milk (12)        | 9 (75) | 10 (83.3) | 3 (25) | 8 (66.6) | 9 (75) | 9 (75) | 2 (16.6) | 2 (16.6) | 5 (25) | 3 (25) |
| Camel raw milk (8)           | 6 (75) | 8 (100) | 1 (12.5) | 7 (87.5) | 8 (100) | 6 (75) | – | – | 2 (25) | 1 (12.5) |
| Donkey raw milk (−)          | – | – | – | – | – | – | – | – | – | – |
| **Total milk (103)**         | **70 (67.9)B** | **83 (80.5)A** | **18 (17.4)ab** | **71 (68.9)C** | **76 (73.7)C** | **65 (63.1)A** | **13 (12.6)ac** | **11 (10.6)abc** | **31 (30)1** | **21 (20.3)a** |
| Traditional cheese (30)      | 23 (76.6) | 26 (86.6) | 6 (20) | 22 (73.3) | 25 (83.3) | 21 (70) | 6 (30) | 5 (16.6) | 15 (50) | 7 (23.3) |
| Traditional cream (15)       | 10 (66.6) | 13 (86.6) | 3 (20) | 11 (73.3) | 12 (80) | 10 (66.6) | 2 (13.3) | 2 (33) | 6 (40) | 4 (26.6) |
| Traditional butter (5)       | 3 (60) | 5 (100) | – | 2 (40) | 2 (40) | 2 (40) | – | – | 1 (20) | 1 (20) |
| Traditional ice cream (27)   | 20 (74.0) | 25 (92.5) | 5 (18.5) | 21 (77.7) | 23 (85.1) | 19 (70.3) | 4 (14.8) | 5 (18.5) | 8 (29.6) | 5 (18.5) |
| **Total dairy products (77)** | **56 (72.7)C** | **69 (89.6)A** | **14 (18.1)c** | **56 (72.7)C** | **62 (80.5)B** | **52 (67.5)A** | **12 (15.5)b** | **12 (15.5)abc** | **30 (38.9)1** | **17 (22)a** |
| **Total (180)**              | **126 (70)** | **152 (84.4)A** | **32 (17.7)ac** | **127 (70.5)C** | **138 (76.6)B** | **117 (65)** | **25 (13.8)1** | **23 (12.7)abc** | **61 (38.8)1** | **38 (21.1)ab** |

METR5: metronidazole (5 μg/disk); AM10: ampicillin (10 μg/disk); CLRT2: clarithromycin (2 μg/disk); ERT5: erythromycin (5 μg/disk); TE30: tetracycline (30 μg/disk); AMX10: amoxicillin (10 μg/disk); FZL1: furazolidone (1 μg/disk); Lev5: levofloxacin (5 μg/disk); TRP25: trimethoprim (25 μg/disk); Cef30: cefsulodin (30 μg/disk).

**The same small and capital letters in rows have significant differences of p <0.05.**
milk samples of Iranian herds were 1.41%, 12.2%, 8.7%, 23.4% and 3.6%, respectively. In a study carried out in Italy, \textit{H. pylori} was detected in 50%, 33%, and 25.6% of raw bovine, sheep, and goat milk, respectively [21]. In Japan, a study detected \textit{H. pylori} in 72.2% of raw cow milk samples [22]. Total distribution of \textit{H. pylori} in milk samples of Greek [23] and American [4] herds were 20% and 60%, respectively. Recent clinical investigation among Iranian cows showed that 16% of milk and 40% of feces samples of seropositive herds tested positive for \textit{H. pylori} [6].

Regarding dairy products, 30% of cheese samples were positive for \textit{H. pylori}. The temperature required for cheesemaking is mainly low. In traditional conditions, in order to create a better clot, the average temperature is less than 30°C. In such circumstances and given the low quality of primary milk and unsanitary conditions of dairy products processing, high levels of \textit{H. pylori} contamination are not unimaginable. The transformation of \textit{H. pylori} into coccoid forms may be another explanation for the high incidence of bacteria in the observed conditions that include acidic environment and high temperature.

Substantial discrepancy in the prevalence of \textit{H. pylori} in different studies could be related to variations in the type of tested sample, number of samples, sampling method, experimental methodology, geographical area, and climate differences in the regions where the samples were collected.

There is indirect hypothesis of \textit{H. pylori} transmission through milk, similar to that obtained for water, but less extensive [4,6,22-24]. These studies led to the hypothesis that \textit{H. pylori} infection can be considered a zoonosis, which is further reinforced by the occurrence of this bacterium in the gastric mucosa of calves, pigs, and horses and its isolation from gastric tissue and milk of sheep [4]. Such findings suggest that these animal species may act as reservoirs and spreaders of \textit{H. pylori}. Moreover, Moniatak et al. [7] showed that \textit{vacA} s1a/m1a was frequently found in \textit{H. pylori} isolated in clinical samples of cow, sheep and humans. They showed 3.4 to 8.4% variability and 92.9 to 98.5% homology between sheep and human samples [7].

Unfortunately, there is no previously published data on the presence of \textit{H. pylori} in dairy products. Among the main reasons for the presence of the bacteria in dairy products are two factors: primary contamination of milk and cross contamination. Use of contaminated water during dairy products processing, handling contamination, use of contaminated equipment and finally lack of public and individual hygiene. Food safety regulations as well as quality standards – including good agricultural practices (GAPs), good manufacturing practices (GMPs), and hazard analysis and critical control points (HACCP) – should be introduced in Iranian food units in order to control contamination and proliferation of pathogenic bacteria.

Close association of \textit{cagA}, \textit{vacA}, \textit{iceA} and \textit{oipA} virulence factors with interleukin 8 (IL-8) production, cytoxin production, gastric epithelial cells adhesion, inflammatory effects, vacuolization and apoptosis in gastric epithelial cells has been previously observed [25-28]. High prevalence of \textit{cagA}, \textit{vacA}, \textit{iceA} and \textit{oipA} virulence factors in cases of gastritis, peptic ulcer and gastric cancer has been reported in the United States, Turkey, Japan and Brazil [29-32]. Since \textit{H. pylori} isolates in our study harbored \textit{cagA} (76.6%) and \textit{vacA} (75%) genes, consumption of milk and dairy products contaminated with virulent strains may provoke duodenal ulceration, gastric mucosal atrophy and gastric cancer.

Another important finding of our investigation was the high presence of antibiotic resistance among \textit{H. pylori} strains isolated from milk and dairy products. Thyagarajan \textit{et al.} [33] and Secka \textit{et al.} [34] reported that \textit{H. pylori} strains from clinical samples had the highest levels of resistance against metronidazole, amoxicillin, ampicillin and tetracycline, which was similar to our results. In a study conducted in India, resistance of \textit{H. pylori} strains against metronidazole, clarithromycin and amoxicillin were 77.9%, 44.7% and 32.8%, respectively [33]. Similar results were reported by Bang \textit{et al.} [35], who found that \textit{H. pylori} isolates were highly resistant to metronidazole (34.7%), clarithromycin (16.7%) and amoxicillin (11.8%). The low antibiotic resistance of \textit{H. pylori} against levofloxacin, furazolidone, clarithromycin, trimethoprim, and cefsulodin may be due to the less frequent prescription of these antibiotics. A possible explanation for the augmented incidence of antibiotic resistance in our study is the indiscriminate and unnecessary veterinary use of these antibiotics.

\textbf{Conclusions}

In Iran, milk and dairy products samples were found to harbor \textit{H. pylori} strains that possess the virulence genes \textit{vacA}, \textit{cagA}, \textit{oipA} and \textit{iceA}. Therefore, consumption of raw milk and their derived products by humans may be the source of \textit{H. pylori} infections. Our findings should raise awareness on \textit{H. pylori} resistance to antibiotics in the country. Clinicians should be cautious when prescribing antibiotics, since metronidazole, ampicillin, erythromycin, tetracycline and amoxicillin are not recommended for treating \textit{H. pylori} infections. Based on the present results, we suggest the prescription of clarithromycin, furazolidone, levofloxacin, trimethoprim and cefsulodin.

\textbf{Abbreviations}

PCR: Polymerase chain reaction; SPSS: Statistical package for the social sciences; \textit{vacA}: Vacuolating cytoxin; \textit{cagA}: Cytotoxin-associated gene; \textit{iceA}: Induced by contact with epithelium; \textit{oipA}: Outer inflammatory protein.

\textbf{Competing interests}

The authors declare that they have no competing interests.
Authors' contribution
Sample collection, DNA extraction and molecular genetic studies were performed by SM, FSD participated in primer sequence alignment, writing and drafting of the manuscript. All authors read and approved the final manuscript.

Acknowledgements
The authors would like to thank Prof H. Momtaz and Prof. A. Shakerian at the Biotechnology Research Center of the Islamic Azad University of Shahrekord for their important technical and clinical support. This work was supported by the Islamic Azad University, Shahrekord Branch, Iran, grant n. 90/0025.

Author details
1 Young Researchers and Elites Club, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran. 2 Department of Food Hygiene, College of Veterinary Medicine, Islamic Azad University, Shahrekord Branch, PO Box 166, Shahrekord, Iran.

Received: 10 July 2014 Accepted: 18 November 2014 Published: 4 December 2014

References
1. Malaty HM: Epidemiology of Helicobacter pylori infection. Best Pract Res Clin Gastroenterol 2007, 21(2):205–214.
2. World Health Organization, International Agency for Research on Cancer. The Globalcancer project, 2010. [http://www.iarc.fr/en/media-centre/sarcnews/index.php?year=2010]
3. Siegel R, Ma J, Zou Z, Jemal A: Cancer statistics, 2014. CA - Cancer J Clin 2014, 64(1):9–29.
4. Dore MP, Sepulveda AR, El-Zimaty H, Yamaoka Y, Osato MS, Mototsugu K, Nieddu AM, Realini G, Graham DY: Isolation of Helicobacter pylori from sheep-implications for transmission to humans. Am J Gastroenterol 2001, 96(10):3361–1401.
5. Rahimi E, Kheirabadi EK: Detection of Helicobacter pylori in bovine, buffalo, camel, ovine, and caprine milk in Iran. Foodborne Pathog Dis 2012, 9(5):453–456.
6. Safaei GH, Rahimi E, Zandib A, Rashidipour A: Helicobacter pylori as a zoonotic infection: the detection of H. pylori antigens in the milk and faeces of cows. J Res Med Sci 2011, 16(2):184–187.
7. Mornat H, Dubii H, Souud N, Golmani M: Study of Helicobacter pylori genotype status in cows, sheep, goats and human beings. BMC Gastroenterol 2010, 14:651. doi:10.1186/1471-230X-14-61.
8. Fan XG, Chua A, Li TG, Zeng QS: Survival of Helicobacter pylori in milk and tap water. J Gastroenterol Hepatol 1998, 13(11):1096–1098.
9. Megraud F: H. pylori antibiotic resistance: prevalence, importance, and advances in testing. Gut 2004, 53(9):1374–1384.
10. Yamaoka Y: Mechanisms of disease: Helicobacter pylori virulence factors. Nat Rev Gastroenterol Hepatol 2010, 7(11):629–641.
11. Kalali B, Mejias-Luque R, Javaheri R, Gerhard M: H. pylori virulence factors: influence on immune system and pathology. Mediat Inflamm 2014, 2014:1–9.
12. Yamaoka Y, Kwon DH, Graham DY: A M(r) 34,000 proinflammatory outer membrane protein (OipA) of Helicobacter pylori. Proc Natl Acad Sci U S A 2000, 97(13):7533–7538.
13. Zheng XY, Hua J, Yeoh KG, Ho B: Association of peptic ulcer with increased expression of Lewis antigens but not cagA, iceA1, and vacA in Helicobacter pylori isolates in an Asian population. Gut 2000, 47(1):18–22.
14. International Dairy Federation (IDF): Milk and Milk Products—Guidance on Sampling, IDF Standard 50C. Brussels, Belgium: International Dairy Federation, 1995.
15. Pons RE, Tatini SR: Survival of Helicobacter pylori in ready-to-eat foods at 4 degrees C. Int J Food Microbiol 2001, 63(1):281–286.
16. Dunn BE, Cohen H, Blaser MJ: Helicobacter pylori. Clin Microbiol Rev 1997, 10(4):720–741.
17. Clinical and Laboratory Standards Institute (CLSI): Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Second Informational Supplement. Document M100-S21, Volume 32. Wayne: Clinical and Laboratory Standards Institute, 2012.
18. Ho SA, Hoyle JA, Lewis FA, Seeker AD, Cross D, Mapstone NP, Dixon MF, Wyatt JI, Tompkins DS, Taylor GR: Direct polymerase chain reaction test for detection of Helicobacter pylori in humans and animals. J Clin Microbiol 1991, 29(11):2543–2549.
19. Venalovic J, Koeuth T, Lupish JR: Distribution of repetitive DNA sequences in Euabacteria and application to fingerprinting of bacterial genomes. Nucleic Acids Res 1991, 19(24):6823–6831.
20. Essawi T, Hannoumdeh W, Sabri I, Swedian W, Faraj MA: Determination of Helicobacter pylori virulence genes in gastric biopsies by PCR. Gastroenterology 2013, 2013:1–4.
21. Quaglia NC, Dambrosio A, Normanno G, Parisi A, Patrono R, Ranieri G, Rezza A, Celano GV: High occurrence of Helicobacter pylori in raw goat, sheep and cow milk confirmed by glmM gene: a risk of food-borne infection? Int J Food Microbiol 2008, 124(1):43–47.
22. Fujimura S, Kawamura T, Kato S, Tateno H, Watanabe A: Detection of Helicobacter pylori in cow’s milk. Lett Appl Microbiol 2002, 35(6):504–507.
23. Angelidis AS, Tirodimos I, Bobos M, Kalamaki MS, Papageorgiou DK, Avantidou M: Detection of Helicobacter pylori in raw bovine milk by fluorescence in situ hybridization (FISH). Int J Food Microbiol 2011, 151(2):252–256.
24. Quaglia NC, Dambrosio A, Normanno G, Celano GV: Evaluation of a Nested-PCR assay based on the phosphoglucomutase mutase gene (glmM) for the detection of Helicobacter pylori from raw milk. Food Control 2009, 20(2):119–123.
25. Baser MJ, Perez-Perez GI, Kleanthous H, Cover TL, Peek RM, Chyou PH, Stemmermann GN, Nomura A: Infection with Helicobacter pylori strains possessing caga is associated with an increased risk of developing adenocarcinoma of the stomach. Cancer Res 1995, 55(18):2111–2115.
26. Jenks P, Megraud F, Labigne A: Clinical outcome after infection with Helicobacter pylori does not appear to be reliably predicted by the presence of any of the genes of the cag pathogenicity island. Gut 1998, 43(8):752–759.
27. Blanke SP: Micro-managing the executioner: pathogen targeting of mitochondria. Trends Microbiol 2005, 13:2564–2571.
28. Yamaoka Y, Reddy R, Graham DY: Helicobacter pylori virulence factor genotypes in children in the United States: clues about genotype and outcome relationships. J Clin Microbiol 2010, 48(7):2550–2551.
29. Nogueira C, Figueiredo C, Carneiro F, Gomes AT, Barreira R, Figueira P, Salgado C, Belo L, Peixoto A, Bravo JC, Bravo LE, Realpe JL, Plassier AP, Quint WG, Ruiz B, Corea P, van Doom LJM: Helicobacter pylori genotypes may determine gastric histopathology. Am J Pathol 2001, 158(2):647–654.
30. Nagyev T, Yula E, Abay G, Koks F: Prevalence and genotypes of Helicobacter pylori in gastric biopsy specimens from patients with gastroduodenal pathologies in the Çukurova region of Turkey. J Clin Microbiol 2009, 47(12):4150–4153.
31. Sasaki T, Hirai H, Izurieta R, Hsiao K, Esterbre E, Salanida A, Calzada J, Fujimoto S, Yamamoto Y: Analysis of Helicobacter pylori genotype in stool specimens of asymptomatic people. Lab Med J 2009, 40(7):412–414.
32. Pereira WN, Ferraz MA, Zabaglia LM, de Labio RW, Ocini WA Branchi Ximenez JP, Neto AC, Pajao SLM, Rasmussen LT: Association among H. pylori virulence markers dupA, caga and vacA in Brazilian patients. J Venom Anim Toxins Incl Trop Dis 2014, 20(1):1–3.
33. Thyagarajan SP, Ray P, Das BK, Ayagiara A, Khan AA, Sharma P, Rao LA, Rajasambandam P, Ramathilagam B, Bhaskar Sharma MP, Naik SR, Habibullah CM: Geographical difference in antimicrobial resistance pattern of Helicobacter pylori clinical isolates from Indian patients: multicentric study. J Clin Microbiol Hepatol 2013, 18(2):1737–1738.
34. Sena G, Berg DE, Antonio M, Corah T, Tagpun M, Walton R, Thomas V, Galano JI, Sancho J, Adsgebola RA, Thomas JE: Antimicrobial susceptibility and resistance patterns among Helicobacter pylori strains from the Gambia, West Africa. Antimicrob Agents Chemother 2013, 57(12):3123–1237.
35. Bang SY, Han DS, Eun CS, Kim JE, Ahn SB, Sohn JH, Jeon YC, Kang JG: Changing patterns of antibiotic resistance of Helicobacter pylori in patients with peptic ulcer disease. Korean J Gastroenterol 2007, 50(6):356–362 [Article in Korean].