VacA and *cagA* genotypes status and antimicrobial resistance properties of *Helicobacter pylori* strains isolated from meat products in Isfahan province, Iran

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Summary

Although *Helicobacter pylori* has a significant impact on the occurrence of severe clinical syndromes, its exact ways of transmission and origin have not been identified. According to the results of some previously published articles, foods with animal origins play a substantial role in the transmission of *H. pylori* to humans. The present investigation was carried out to study the vacuolating cytotoxin A (*vacA*) and cytotoxin associated gene A (*cagA*) genotypes status and antibiotic resistance properties of *H. pylori* strains recovered from minced-meat and hamburger samples. A total of 150 meat product samples were collected from supermarkets. All samples were cultured and the susceptive colonies were then subjected to nested-PCR, PCR-based genotyping and disk diffusion methods. 11 out of 150 samples (7.33%) were positive for *H. pylori*. All the isolates were further identified using the nested-PCR assay. Prevalence of *H. pylori* in hamburger and minced-meat samples was 1.42% and 12.5%, respectively. *S1a*, *m1a* and *cagA* were the most commonly detected genotypes. The most commonly detected combined genotypes in the *H. pylori* strains of minced-meat were *s1am1a* (10%), *s1am1b* (10%) and *s2m1a* (10%). *Helicobacter pylori* strains of meat products harbored the highest levels of resistance against ampicillin (90.90%), erythromycin (72.72%), amoxicillin (72.72%), trimethoprim (63.63%), tetracycline (63.63%), and clarithromycin (63.63%). Hamburger and minced-meat samples may be the sources of virulent and resistant strains of *H. pylori*. Meat products are possible sources of resistant and virulent strains of *H. pylori* similar to those *vacA* and *cagA* genotypes. Using healthy raw materials and observation of personal hygiene can reduce the risk of *H. pylori* in meat products.

Key words: Antibiotic resistance pattern, *cagA*, *Helicobacter pylori*, Meat products, *vacA*

Introduction

In spite of the high nutritional value of meat and its products (Valsta *et al*., 2005; Pereira and Vicente, 2013), their production and processing have low hygienic conditions in Iran. Therefore, many kinds of infections and food poisonings can occur due to their consumption.

Helicobacter pylori is a microaerophilic, Gram-negative spiral bacterium which is classically considered as a main cause of duodenal ulcer, peptic ulcer disease, gastric adenocarcinoma, type B gastritis and lymphoma allaround the world (Dunn *et al*., 1997; Van Leerdam and Tytgat, 2002; Kusters *et al*., 2006). Human stomach is considered as a main reservoir of *H. pylori* (Dunn *et al*., 1997; Van Leerdam and Tytgat, 2002; Kusters *et al*., 2006) but according to the previous hypothesis and results of some published investigations, foods with animal origins play an important role in the distribution and transmission of *H. pylori* (Van Duynhoven and Jonge, 2001; Brown, 2000; Herrera 2004). Appropriate circumstances such as pH, activated water (AW), moisture and temperature cause *H. pylori* to easily survive in foods with animal origin and especially meat and meat products (Brown, 2000; Herrera, 2004; Atapoor *et al*., 2014).

Genotyping using gold genetic markers like vacuolating cytotoxin A (*vacA*) and cytotoxin associated gene A (*cagA*) is an appropriate approach to study the exact correlations between *H. pylori* isolates from different sources (Momtaz *et al*., 2012, 2014; Yahaghi *et al*., 2014). *VacA* gene is polymorphic and containing of mutable signal regions (type *s1* or *s2*) and also mid-regions (type *m1* or *m2*). The *s1* type is additionally subtyped into *s1a*, *s1b* and *s1c* and the *m1* into *m1a* and *m1b* alleles (Momtaz *et al*., 2012, 2014; Yahaghi *et al*., 2014). *Cag* pathogenicity island (PAI) has been shown to be complicated in persuading ulceration, inflammation and carcinogenesis (Momtaz *et al*., 2012, 2014; Yahaghi *et al*., 2014).

Treatment is a precarious point in the epidemiology of *H. pylori* in humans, animals and food products, since therapeutic options have become somewhat restricted because of the presence of multidrug resistant strains of this bacterium (De Francesco *et al*., 2010; Talebi Bezmin Abadi *et al*., 2010; Mousavi *et al*., 2014). Prevalence of *H. pylori* resistance against various types of antibiotics were between 5 and 100% (De Francesco *et al*., 2010; Talebi Bezmin Abadi *et al*., 2010; Mousavi *et al*., 2014) which were considerably high.

There were no previously published data on the

presence of *H. pylori* in foods and especially meat products all-around (Brown, 2000; Van Duynhoven and Jonge, 2001; Herrera, 2004; Rahimi and Kheirabadi, 2012; Atapoor *et al*., 2014). Therefore, the present investigation was carried out to genotype and study the antibiotic resistance properties of *H. pylori* strains isolated from hamburger and minced-meat samples of Iranian supermarkets.

Materials and Methods

Sample collection

From October 2015 to January 2016, a total of 150 meat product samples including hamburger (n=80) and minced-meat (n=70) were collected from supermarkets of various parts of Isfahan province, Iran. Samples (100 mg, in sterile glass containers) were transported to the laboratory at 4°C using ice-packs.

Isolation of *H. pylori*

Twenty five g of each homogenized sample was added to 225 ml of Wilkins Chalgren anaerobe broth (Oxoid, UK) supplemented with colistin methane sulfonate (30 mg/L), 5% of horse serum (Sigma, St. Louis, MO, USA), nalidixic acid (30 mg/L), vancomycin (10 mg/L), cycloheximide (100 mg/L), and trimethoprim (30 mg/L) (Sigma, St. Louis, MO, USA) and incubated for 7 days at 37°C with shaking under microaerophilic conditions (5% oxygen, 85% nitrogen and 10% CO_2) using MART system (Anoxamat, Lichtenvoorde, The Netherlands). 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 methanesulfonate, 100 mg/L cycloheximide, 30 mg/L nalidixic acid, 30 mg/L trimethoprim, and 10 mg/L vancomycin (Sigma, St. Louis, MO, USA) and incubated for 7 days at 37°C under microaerophilic conditions (5% oxygen, 85% nitrogen and 10% CO_2) using MART system (Anoxamat, Lichtenvoorde, The Netherlands). For comparison, a reference strain of *H. pylori* (ATCC 43504) was employed.

Antimicrobial susceptibility testing

The pure cultures of *H. pylori* 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 (Merck, Germany) supplemented with 5% defibrinated sheep blood and 7% fetal calf serum, according to the Clinical Laboratory Standards Institute (Wayne, 2012). The antimicrobial resistance of *H. pylori* was measured against the widely used antibiotics in cases of *H. pylori* gastric ulcer. The following antimicrobial disks (HiMedia Laboratories, Mumbai, India) were used: ampicillin (10 µg), metronidazole (5 µg), erythromycin (5 µg), clarithromycin (2 µg), amoxicillin (10 µg), tetracycline (30 µg), levofloxacin (5 µg), streptomycin (10 µg), rifampin (30 µg), cefsulodin (30 µg), trimethoprim (25 µg), furazolidone (1 µg) and spiramycin (100 µg). After incubation at 37°C for 48 h in a microaerophilic atmosphere (5% oxygen, 85% nitrogen and 10% CO_2) using MART system (Anoxamat, 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 (2012) (Wikler, 2006). The *H. pylori* ATCC 43504 was used as quality control organisms in the antimicrobial susceptibility determination.

DNA extraction and nested-PCR assay

Typical colonies of *H. pylori* were further identified using the nested-PCR method. Genomic DNA was extracted from the typical colonies using a DNA extraction kit for cells and tissues (Fermentas, Germany) according to the manufacturer's instructions and its density was assessed by optic densitometry. The first and second step of PCR was performed based on the method described previously (Yamada *et al*., 2008). List of primers and nested-PCR conditions is shown in Table 1.

Genotyping of *vacA* and *cagA* genes of *H. pylori*

Presence of the *vacA* and *cagA* alleles was determined using PCR technique. List of primers is shown in Table 2 (Chomvarin *et al*., 2008; Mansour *et al*., 2010). All PCR reactions were done using the programmable thermal cycler (Eppendorf Co., Germany). All runs included one negative DNA control consisting of PCR grade water and two or more positive controls (26695, J99, SS1, Tx30, 88-23 and 84-183).

Gel electrophoresis

The PCR amplification products (10 µL) were

Table 1: Oligonucleotide primers and reaction conditions used for confirmation of *H. pylori* strains of meat products using nested-PCR method

Target gene	Primer sequence (5´-3´)	Size of product (bp)	Volume of PCR reaction (50 µL)	Annealing temperature	References
EHC-U EHC-L	F: CCCTCACGCCATCAGTCCCAAAAA R: AAGAAGTCAAAAACGCCCCAAAAC	417	5 µL PCR buffer 10X 5 µL dNTP (Fermentas) 3 µL of each primers F & R 0.3 µL Taq DNA polymerase (Fermentas) 5 µL DNA template 31.7 µL distilled water	55°C ---------- 120 s	Yamada *et al*. (2008)
ET-5U ET-5L	F: GGCAAATCATAAGTCCGCAGAA R: TGAGACTTTCCTAGAAGCGGTGTT	230	5 µL PCR buffer 10X 5 µL dNTP (Fermentas) 3 µL of each primers F & R 0.3 µL Taq DNA polymerase (Fermentas) 5 µL of first-step PCR product 31.7 µL distilled water	55°C ---------- 120 s	Yamada *et al*. (2008)

Table 2: Oligonucleotide primers, volume and programs of PCR reactions used for genotyping of *vacA* and *cagA* alleles of *H. pylori* strains of meat products

Genes	Primer sequence (5´-3´)	Size of product (bp)	Volume of PCR reaction (50 µL)	Annealing temperature	References
vacA s1a	F: CTCTCGCTTTAGTAGGAGC	213	5 µL PCR buffer 10X	64°C ---------- 50 s	Chomvarin *et al.* (2008), Mansour *et al.* (2010)
	R: CTGCTTGAATGCGCCAAAC		1.5 mM $Mgcl_2$		
vacA s1b	F: AGCGCCATACCGCAAGAG	187	200 µM dNTP (Fermentas)		
	R: CTGCTTGAATGCGCCAAAC		0.5 µM of each primers F & R		
vacA s1c	F: CTCTCGCTTTAGTGGGGYT	213	1.25 U Taq DNA polymerase (Fermentas)		
	R: CTGCTTGAATGCGCCAAAC		2.5 µL DNA template		
vacA s2	F: GCTAACACGCCAAATGATCC	199			
	R: CTGCTTGAATGCGCCAAAC				
vacA m1a	F: GGTCAAAATGCGGTCATGG	290			
	R: CCATTGGTACCTGTAGAAAC				
vacA m1b	F: GGCCCCAATGCAGTCATGGA	291			
	R: GCTGTTAGTGCCTAAAGAAGCAT				
vacA m2	F: GGAGCCCCAGGAAACATTG	352			
	R: CATAACTAGCGCCTTGCA				
cagA	F: GATAACAGCCAAGCTTTTGAGG	300	5 µL PCR buffer 10X	56°C ---------- 60 s	Chomvarin *et al.* (2008), Mansour *et al.* (2010)
	R: CTGCAAAAGATTGTTTGGCAGA		2 mM $Mgcl_2$		
			150 µM dNTP (Fermentas)		
			0.75 µM of each primers F & R		
			1.5 U Taq DNA polymerase (Fermentas)		
			3 µL DNA template		

subjected to electrophoresis in a 2% agarose gel in 1X TBE buffer at 80 V for 30 min, stained with SYBR Green, and images were obtained in a UVIdoc gel documentation system (UK). The PCR products were identified by 100 bp DNA size marker (Fermentas, Germany).

Statistical analysis

Data were transferred to Microsoft Excel spreadsheet (Microsoft Corp., Redmond, WA, USA) for analysis. Using SPSS 16.0 statistical software (SPSS Inc., Chicago, IL, USA), Chi-square test and Fisher's exact two-tailed test analysis were performed and differences were considered significant at values of $P<0.05$. Distribution of *H. pylori* genotypes and antimicrobial resistance properties were statistically analyzed.

Results

Distribution of *H. pylori*

Table 3 shows the total distribution of *H. pylori* in the meat product samples. Of 150 samples collected, 11 samples (7.33%) were positive for *H. pylori*. All the isolates were further identified using the nested-PCR technique (Fig. 1). Prevalence of *H. pylori* in hamburger and minced-meat samples were 1.42% and 12.5%, respectively. A significant difference for the prevalence of *H. pylori* was shown between hamburger and minced-meat ($P<0.05$).

Genotyping pattern of the *H. pylori* isolates

Table 4 presents the total prevalence of *vacA* and *cagA* genotypes in *H. pylori* strains of meat products. *S1a* is the only positive allele for the *H. pylori* strains of hamburger. The most commonly detected genotypes in the *H. pylori* strains of minced-meat were *sla* (20%), *mla* (20%) and *cagA* (20%). Statistical significant difference was seen between the prevalence of genotypes of types of samples ($P<0.05$). Table 5 indicates the total prevalence of combined genotypes in the *H. pylori* strains of meat products. There were no detected combined genotypes in the *H. pylori* strains of hamburger. The most commonly detected combined

Table 3: Total prevalence of *H. pylori* in meat products

Type of samples	No. samples collected	No. *H. pylori* in culture (%)	No. *H. pylori* confirmed in nested-PCR (%)
Hamburger	70	1 (1.42)	1 (1.42)
Minced-meat	80	10 (12.5)	10 (12.5)
Total	150	11 (7.33)	11 (7.33)

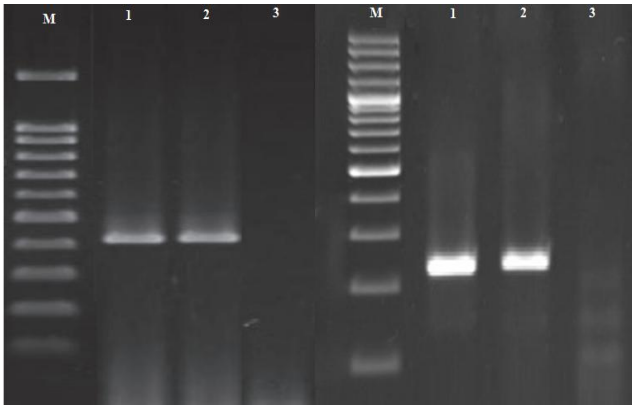

Fig. 1: Electrophoresis of PCR products in nested-PCR reaction (the left figure is the first PCR and the right figure is the second PCR reactions). M: 100 bp ladder. Line 1: Positive sample, Line 2: Positive control, and Line 3: Negative control

Table 4: Total prevalence of *vacA* and *cagA* genotypes in *H. pylori* strains of meat products

Type of samples (No. positive)	Distribution of genotypes (%)							
	VacA							*CagA*
	S1a	*S1b*	*S1c*	*S2*	*M1a*	*M1b*	*M2*	
Hamburger (1)	1 (100)	-	-	-	-	-	-	-
Minced-meat (10)	2 (20)	1 (10)	1 (10)	1 (10)	2 (20)	1 (10)	-	2 (20)
Total (11)	3 (27.27)	1 (9.09)	1 (9.09)	1 (9.09)	2 (18.18)	1 (9.09)	-	2 (18.18)

Table 5: Total prevalence of combined genotypes of *H. pylori* isolated from meat products

Type of samples (No. positive)	Distribution of combined genotypes (%)											
	s1am1a	*s1am1b*	*s1am2*	*s1bm1a*	*s1bm1b*	*s1bm2*	*s1cm1a*	*s1cm1b*	*s1cm2*	*s2m1a*	*s2m1b*	*s2m2*
Hamburger (1)	-	-	-	-	-	-	-	-	-	-	-	-
Minced-meat (10)	1 (10)	1 (10)	-	-	-	-	-	-	-	1 (10)	-	-
Total (11)	1 (9.09)	1 (9.09)	-	1 (9.09)	-	-	-	-	-	1 (9.09)	-	-

Table 6: Antimicrobial resistance properties of *H. pylori* strains isolated from meat products

Types of samples (No. positive results)	Pattern of antibiotic resistance (%)												
	AM10*	Met5	ER5	CLR2	AMX10	Tet30	Lev5	S10	RIF30	Cef30	TRP25	FZL1	Spi100
Hamburger (1)	1 (100)	-	1 (100)	1 (100)	1 (100)	1 (100)	-	-	-	-	1 (100)	-	-
Minced-meat (10)	9 (90)	3 (30)	7 (70)	6 (60)	7 (70)	6 (60)	5 (50)	1 (10)	1 (10)	1 (10)	6 (60)	1 (10)	1 (10)
Total (11)	10 (90.90)	3 (27.27)	8 (72.72)	7 (63.63)	8 (72.72)	7 (63.63)	5 (45.45)	1 (9.09)	1 (9.09)	1 (9.09)	7 (63.63)	1 (9.09)	1 (9.09)

* AM10: Ampicillin (10 µg), Met5: Metronidazole (5 µg), ER5: Erythromycin (5 µg), CLR2: Clarithromycin (2 µg), AMX10: Amoxicillin (10 µg), Tet30: Tetracycline (30 µg), Lev5: Levofloxacin (5 µg), S10: Streptomycin (10 µg), RIF30: Rifampin (30 µg), Cef30: Cefsulodin (30 µg), TRP25: Trimethoprim (25 µg), FZL1: Furazolidone (1 µg), and Spi100: Spiramycin (100 µg)

genotypes in the *H. pylori* strains of minced-meat were *s1am1a* (10%), *s1am1b* (10%) and *s2m1a* (10%).

Antibiotic resistance pattern of *H. pylori* strains

Table 6 represents the results of the antibiotic resistance pattern of the *H. pylori* strains of meat products. We found that the *H. pylori* isolates of meat products harbored the highest levels of resistance against ampicillin (90.90%), erythromycin (72.72%), amoxicillin (72.72%), trimethoprim (63.63%), tetracycline (63.63%) and clarithromycin (63.63%). Statistically significant difference was seen between the types of meat product samples and prevalence of antibiotic resistance ($P<0.05$).

Discussion

Results of present investigations revealed that resistant and virulent strains of *H. pylori* can survive in hamburger and especially minced-meat samples of the Iranian retail markets. It was shown that contaminated hamburger and minced-meat are important vectors for transmission of resistant and virulent strains of *H. pylori* to humans. We found that 7.33% of all the samples were positive for *H. pylori*. Although the main reason for considerable prevalence of *H. pylori* in meat product samples is unknown, the roles of secondary routes of contamination should not be overlooked. High presence of human-based genotypes (*s1am1a*, *s1am1b*, *s2m1a* and …) and also resistance of bacterial isolates against human-based antibiotics (amoxicillin, erythromycin, trimethoprim, clarithromycin and …) can indirectly support the role of cross contaminations of meat product samples. It seems that meat washing, storing, transporting, processing and packaging are the main stages which may increase the prevalence of *H. pylori* contamination. Transmission of *H. pylori* strains from the hands and also droplets of saliva of infected staff of factories and also butchers is another reason for high prevalence of *H. pylori*.

Several investigations have been done in the fields of *H. pylori* and food contamination allaround the world. Prevalence of *H. pylori* in vegetable and salad (Yahaghi *et al*., 2014), milk (Rahimi and Kheirabadi, 2012) dairy products (Mousavi *et al*., 2014), vegetable (Atapoor *et al*., 2014) and restaurant foods (Yahaghi *et al*., 2014) of previous investigations was 10.86%, 12.50%, 19.2%, 13.68% and 14%, respectively which were higher than our results. Several studies which have been focused on the prevalence of *H. pylori* in foods with animal origins (Dore *et al*., 2001; Quaglia *et al*., 2008; Angelidis *et al*., 2011; Rahimi and Kheirabadi, 2012) indicated that the prevalence of bacteria in these types of foods ranged from 20 to 73%. Mhaskar *et al*. (2013) showed that meat consumption (OR: 2.35, 95% CI: 1.30-4.23), eating restaurant food (OR: 3.77, 95% CI: 1.39-10.23) and drinking nonfiltered or non-boiled water (OR: 1.05, 95% CI: 1.01-1.23) were the main risk factors for *H. pylori* infection which can indirectly support the results of our investigation about considerable prevalence in meat. Webberley *et al*. (1993) reported that meat-eater had the highest levels of anti-*H. pylori* IgG compared to vegans, which may indicate the impact of meat-eating as a risk factor for occurrence of the infection.

VacA and *cagA* and especially *s1a*, *m1a*, *s1am1a*, *s1am1b* and *s2m1a* alleles had a considerable prevalence in the *H. pylori* isolates of meat products. There were only four previously published data about the genotyping of *H. pylori* strains of food samples in the literature (Momtaz *et al*., 2014; Mousavi *et al*., 2014; Yahaghi *et al*., 2014; Hemmatinezhad *et al*., 2016; Saeidi and Sheikhshahrokh, 2016). All of these studies were conducted in Iran and were in agreement with our findings. Yahaghi *et al*. (2014) showed that the most commonly detected genotypes in the *H. pylori* strains of vegetable and salad samples were *oipA* (86.44%), *cagA* (57.625), *vacA s1a* (37.28%) and *vacA m1a* (30.50%). Mousavi *et al*. (2014) showed that the most frequently detected genes in the *H. pylori* strains recovered from milk and dairy products were *cagA* (76.60%) and *vacA* (75%). Hemmatinezhad *et al*. (2016) reported that the most frequent genotypes in the *H. pylori* strains of ready to eat foods were *vacA s1a* (78.37%), *vacA m2* (75.67%), *vacA m1a* (51.35%), *cagA* (41.89%), *s1am2* (70.27%), *s1am1a* (39.18%) and *m1am2* (31.08%) were the most prevalent detected combined genotypes. Saeidi and Sheikhshahrokh (2016) revealed that *m1as1a* (68.52%), *m1as1b* (60.40%), *m1bs1b* (55.83%) and *m1bs1a* (53.29%) were the most regularly detected combined genotypes in the *H. pylori* strains of foods with animal origins. High prevalence of *cagA* and *vacA s1a*, *m1a* and *m2* alleles in the *H. pylori* strains of human and animal clinical samples and also foods have been reported

previously (Nagiyev *et al*., 2009; Alikhani *et al*., 2014; Havaei *et al*., 2014; Momtaz *et al*., 2014; Mousavi *et al*., 2014; Yahaghi *et al*., 2014; Miftahussurur *et al*., 2015; Hemmatinezhad *et al*., 2016; Saeidi and Sheikhshahrokh, 2016). Ghorbani *et al*. (2016) revealed that 20% of all types of food samples were contaminated with *H. pylori*. They showed that the most commonly detected genotypes were *vacA s1a*, *vacA m1a* and *vacA m2* and the most commonly determined combined genotypes were *s1am2* (45%), *s1am1a* (40%) and *m1am2* (35%), which was similar to our findings, too.

Among various investigations carried on the antibiotic resistance pattern of *H. pylori* strains of foods, the results of Hemmatinezhad *et al*. (2016) (amoxicillin (94.59%), ampicillin (93.24%), metronidazole (89.18%) and tetracycline (72.97%)), Yahaghi *et al*. (2014) (metronidazole (77.96%), amoxicillin (67.79%), and ampicillin (61.01%)) and Mousavi *et al*. (2014) (ampicillin (84.4%), tetracycline (76.6%), erythromycin (70.5%) and metronidazole (70%)) were similar to our findings. These antibiotics are one of the first choice treatment agents for *H. pylori* infection and the high prevalence of resistance against these antibiotic is due to their irregular, intense and illegal prescription not only for the cases of *H. pylori* infections but also for all types of infectious diseases of the digestive tract. The possibility of considering streptomycin, rifampin, cefsulodin, furazolidone and spiramycin antibiotics as an alternative for treatment of *H. pylori* could be suggested in the cases of infections.

Iranian meat products and especially hamburger and minced-meat harbored resistant strains of *H. pylori* and their pathogenic genotypes. Contaminated meat products may be the sources of the bacteria which can enter to the human population in a period of time. Diversity of *H. pylori* genotypes between various types of samples may indicate that there were various sources of contamination. Meat products are commonly cooked before consumption, however, appropriate conditions of processing including time and temperature are not respected well in some factories producing meat products. Cautious prescription of antibiotics and careful health monitoring in slaughterhouses, butchers and meat product factories such as accurate meat inspection, using high quality raw materials and observation of personal hygiene may reduce the risk of transmission of *H. pylori* to human.

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Conflict of interest

The authors declare that they have no competing interests.

References

Alikhani, MY; Arebestani, MR; Khorasani, MS; Majlesi, A and Jaefari, M (2014). Evaluation of *Helicobacter pylori vacA* and *cagA* genotypes and correlation with clinical outcome in patients with dyspepsia in Hamadan province, Iran. Iran. Red. Crescent. Med. J., 16: e19173.

Angelidis, AS; Tirodimos, I; Bobos, M; Kalamaki, MS; Papageorgiou, DK and Arvanitidou, M (2011). Detection of *Helicobacter pylori* in raw bovine milk by fluorescence in situ hybridization (FISH). Int. J. Food. Microbiol., 151: 252-256.

Atapoor, S; Dehkordi, FS and Rahimi, E (2014). Detection of *Helicobacter pylori* in various types of vegetables and salads. Jundishapur. J. Microbiol., 7: e10013.

Brown, LM (2000). *Helicobacter pylori*: epidemiology and routes of transmission. Epidemiol. Rev., 22: 283-297.

Chomvarin, C; Namwat, W; Chaicumpar, K; Mairiang, P; Sangchan, A; Sripa, B; Tor-Udom, S and Vilaichone, RK (2008). Prevalence of *Helicobacter pylori vacA*, *cagA*, *cagE*, *iceA* and *babA2* genotypes in Thai dyspeptic patients. Int. J. Infect. Dis., 12: 30-36.

De Francesco, V; Giorgio, F; Hassan, C; Manes, G; Vannella, L; Panella, C; Ierardi, E and Zullo, A (2010). Worldwide *H. pylori* antibiotic resistance: a systematic. J. Gastrointestin. Liver Dis., 19: 409-414.

Dore, MP; Sepulveda, AR; El-Zimaity, H; Yamaoka, Y; Osato, MS; Mototsugu, K; Nieddu, AM; Realdi, G and Graham, DY (2001). Isolation of *Helicobacter pylori* from sheep—implications for transmission to humans. Am. J. Gastroenterol., 96: 1396-1401.

Dunn, BE; Cohen, H and Blaser, MJ (1997). *Helicobacter pylori*. Clin. Microbiol. Rev., 10: 720-741.

Ghorbani, F; Gheisari, E and Safarpoor Dehkordi, F (2016). Genotyping of *vacA* alleles of *Helicobacter pylori* strains recovered from some Iranian food items. Trop. J. Pharm. Res., 15: 1631-1636.

Havaei, SA; Mohajeri, P; Khashei, R; Salehi, R and Tavakoli, H (2014). Prevalence of *Helicobacter pylori vacA* different genotypes in Isfahan, Iran. Adv. Biomed. Res., 3: 48.

Hemmatinezhad, B; Momtaz, H and Rahimi, E (2016). *VacA*, *cagA*, *iceA* and *oipA* genotypes status and anti-microbial resistance properties of *Helicobacter pylori* isolated from various types of ready to eat foods. Ann. Clin. Microbiol. Antimicrob., 15: 2.

Herrera, AG (2004). *Helicobacter pylori* and food products: a public health problem. Methods Mol. Biol., 268: 297-301.

Kusters, JG; van Vliet, AH and Kuipers, EJ (2006). Pathogenesis of *Helicobacter pylori* infection. Clin. Microbiol. Rev., 19: 449-490.

Mansour, KB; Fendri, C; Zribi, M; Masmoudi, A; Labbene, M; Fillali, A; Mami, NB; Najjar, T; Meherzi, A and Sfar, T (2010). Prevalence of *Helicobacter pylori vacA*, *cagA*, *iceA* and *oipA* genotypes in Tunisian patients. Ann. Clin. Microbiol. Antimicrob., 9: 10.

Mhaskar, RS; Ricardo, I; Azliyati, A; Laxminarayan, R; Amol, B; Santosh, W and Boo, K (2013). Assessment of risk factors of *Helicobacter pylori* infection and peptic ulcer disease. J. Glob. Infect. Dis., 5: 60-67.

Miftahussurur, M; Sharma, RP; Shrestha, PK; Suzuki, R; Uchida, T and Yamaoka, Y (2015). Molecular epidemiology of *Helicobacter pylori* infection in Nepal: specific ancestor root. Plos. One. 10: e0134216.

Momtaz, H; Dabiri, H; Souod, N and Gholami, M (2014). Study of *Helicobacter pylori* genotype status in cows, sheep, goats and human beings. BMC. Gastroenterol., 14: 61.

Momtaz, H; Souod, N; Dabiri, H and Sarshar, M (2012). Study of *Helicobacter pylori* genotype status in saliva, dental plaques, stool and gastric biopsy samples. World J. Gastroenterol., 18: 2105-2111.

Mousavi, S; Dehkordi, FS and Rahimi, E (2014). Virulence factors and antibiotic resistance of *Helicobacter pylori* isolated from raw milk and unpasteurized dairy products in Iran. J. Venom. Anim. Toxins. Incl. Trop. Dis., 20: 51.

Nagiyev, T; Yula, E; Abayli, B and Koksal, F (2009). Prevalence and genotypes of *Helicobacter pylori* in gastric biopsy specimens from patients with gastroduodenal pathologies in the Cukurova region of Turkey. J. Clin. Microbiol., 47: 4150-4153.

Pereira, PM and Vicente, AF (2013). Meat nutritional composition and nutritive role in the human diet. Meat Sci., 93: 586-592.

Quaglia, N; Dambrosio, A; Normanno, G; Parisi, A; Patrono, R; Ranieri, G; Rella, A and Celano, G (2008). High occurrence of *Helicobacter pylori* in raw goat, sheep and cow milk inferred by *glmM* gene: a risk of food-borne infection? Int. J. Food. Microbiol., 124: 43-47.

Rahimi, E and Kheirabadi, EK (2012). Detection of *Helicobacter pylori* in bovine, buffalo, camel, ovine, and caprine milk in Iran. Foodborne. Pathog. Dis., 9: 453-456.

Saeidi, E and Sheikhshahrokh, A (2016). *VacA* genotype status of *Helicobacter pylori* isolated from foods with animal origin. Biomed. Res. Int., 2016: 1-6.

Talebi Bezmin Abadi, A; Mobarez, AM; Taghvaei, T and Wolfram, L (2010). Antibiotic resistance of *Helicobacter pylori* in Mazandaran, north of Iran. Helicobacter. 15: 505-509.

Valsta, L; Tapanainen, H and Männistö, S (2005). Meat fats in nutrition. Meat Sci., 70: 525-530.

Van Duynhoven, YT and Jonge, RD (2001). Transmission of *Helicobacter pylori*: a role for food? Bull. World Health Org., 79: 455-460.

Van Leerdam, M and Tytgat, G (2002). *Helicobacter pylori* infection in peptic ulcer haemorrhage. Aliment. Pharmacol. Ther., 16(s1): 66-78.

Wayne, P (2012). Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. Twenty-second informational supplement M100-S21. United States.

Webberley, MJ; Webberley, JM; Newell, DG; Lowe, P and Melikian, V (1993). Seroepidemiology of *Helicobacter pylori* infection in vegans and meat-eaters. Epidemiol. Infect., 108: 457-462.

Yahaghi, E; Khamesipour, F; Mashayekhi, F; Safarpoor Dehkordi, F; Sakhaei, MH; Masoudimanesh, M and Khameneie, MK (2014). *Helicobacter pylori* in vegetables and salads: genotyping and antimicrobial resistance properties. Biomed. Res. Int., Article ID 75794.

Yamada, R; Yamaguchi, A and Shibasaki, K (2008). Detection and analysis of *Helicobacter pylori* DNA in the gastric juice, saliva, and urine by nested PCR. Oral. Sci. Int., 5: 24-34.