

Phenotypic and Genotypic Assessment of Antibiotic Resistance and Genotyping of *vacA*, *cagA*, *iceA*, *oipA*, *cagE*, and *babA2* Alleles of *Helicobacter pylori* Bacteria Isolated from Raw Meat

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Background: Foodstuffs with animal origins, particularly meat, are likely reservoirs of *Helicobacter pylori*.

Purpose: An existing survey was accompanied to assess phenotypic and genotypic profiles of antibiotic resistance and genotyping of *vacA*, *cagA*, *cagE*, *iceA*, *oipA*, and *babA2* alleles amongst the *H. pylori* bacteria recovered from raw meat.

Methods: Six-hundred raw meat samples were collected and cultured. *H. pylori* isolates were tested using disk diffusion and PCR identification of antibiotic resistance genes and genotyping.

Results: Fifty-two out of 600 (8.66%) raw meat samples were contaminated with *H. pylori*. Raw ovine meat (13.07%) had the uppermost contamination. *H. pylori* bacteria displayed the uppermost incidence of resistance toward tetracycline (82.69%), erythromycin (80.76%), trimethoprim (65.38%), levofloxacin (63.46%), and amoxicillin (63.46%). All *H. pylori* bacteria had at least resistance toward one antibiotic, even though incidence of resistance toward more than eight antibiotics was 28.84%. Total distribution of *rdxA*, *pbp1A*, *gyrA*, and *cla* antibiotic resistance genes were 59.61%, 51.92%, 69.23%, and 65.38%, respectively. *VacA s1a* (84.61%), *s2* (76.92%), *m1a* (50%), *m2* (39.13%), *iceA1* (38.46%), and *cagA* (55.76%) were the most generally perceived alleles. *Slam1a* (63.46%), *s2m1a* (53.84%), *slam2* (51.92%), and *s2m2* (42.30%) were the most generally perceived genotyping patterns. Frequency of *cagA*-, *oipA*-, and *babA2*- genotypes were 44.23%, 73.07%, and 80.76%, respectively. A total of 196 combined genotyping patterns were also perceived.

Conclusion: The role of raw meat, particularly ovine meat, in transmission of virulent and resistant *H. pylori* bacteria was determined. *VacA* and *cagA* genotypes had the higher incidence. *CagE*-, *babA2*-, and *oipA*- *H. pylori* bacteria had the higher distribution. Supplementary surveys are compulsory to originate momentous relations between distribution of genotypes, antibiotic resistance, and antibiotic resistance genes.

Keywords: *Helicobacter pylori*, antibiotic resistance, genotyping, raw meat

Introduction

Meat of animals, particularly camel, caprine, ovine, bovine, and buffalo species, afford a bundle of nutrient components difficult to gain in diets with incomplete or no meat.¹ Reversely, raw meat is not unavoidably safe, as evidenced by considerable rates of foodborne diseases accompanying with its consumption.² Similarly, several outbreaks of foodborne diseases have been conveyed owing to the consumption of contaminated meat samples.²

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Helicobacter pylori (*H. pylori*) is a microaerophilic and Gram-negative flagellated bacterium responsible for the occurrence of peptic ulcer disease, gastric adenocarcinoma, duodenal ulcer, type B gastritis, mucosa-associated lymphoid tissue (MALT) lymphoma, and gastric B-cell lymphoma.³ The main reservoir of *H. pylori* bacteria is the human stomach.³ In keeping with this, foods with animal origins, particularly meat, may play an imperative portion in transmission of *H. pylori* infections to humans.^{4,5} Foods with animal origins provide appropriate circumstances such as pH, moisture and activated water (AW) contents, and temperature for growth and survival of *H. pylori*.^{4,5} Additionally, the role of meat consumption as a risk factor for occurrence of *H. pylori* infections has been conveyed.^{6,7} Likewise, the bacterium has been recovered from diverse kinds of foods with animal origins.^{6,7}

H. pylori infections are associated with the presence and activity of certain virulence markers such as Vacuolating Cytotoxin A (*vacA*), Cytotoxin Associated Gene A and E (*cagA* and *cagE*), Induced by Contact with the Epithelium Antigen (*iceA*), Outer Inflammatory Protein Antigen (*oipA*), and Blood Group Antigen-Binding Adhesin gene (*babA*).^{8,9} The *vacA* gene is polymorphic, containing mutable signals (type *s1* or *s2*) and mid-regions (type *m1* or *m2*). The *s1* type is further alienated into *s1a*, *s1b* and *s1c* and the *m1* into *m1a* and *m1b* alleles. The *cagA* gene is an indicator for the genomic pathogenicity island of *c.* 40 kb [*cag* pathogenicity island (*cag*-PAI)] and its activity is believed to be cooperated with interleukin 8 secretion, local inflammation, and severe and/or complicated occurrence of peptic ulcers and gastrointestinal disorders.^{8,9} *CagE* gene was found to serve as an improved biomarker of an intact *cag*-PAI in patients with severe gastrointestinal disorders.^{8,9} *BabA2* gene mediates adherence of *H. pylori* to human Lewis b blood-group antigens on gastric epithelial cells.^{8,9} *OipA* is a significant virulence marker which is associated with clinically imperative presentation of peptic ulcers, such as enhanced interleukin-8 secretion and increased inflammation.^{8,9} The *iceA* gene was detected in the *H. pylori* recovered from patients with gastrointestinal disorders.^{8,9} There are at least two alleles of *iceA*., *iceA1*, and *iceA2*.^{8,9} The relationship between *H. pylori iceA1* and *iceA2* and clinical outcomes has been addressed by some researchers.^{8,9} The presence of these alleles has been conveyed in different research conducted on diverse kinds of foods with animal origins.¹⁰ Genotyping using these virulence markers is considered as

one of the best approaches to study the correlations between *H. pylori* isolates from different samples.

Antibiotic therapy is one of the best aspects of treatments of *H. pylori* infections. However, therapeutic choices have become slightly limited owing to the occurrence of resistance in some *H. pylori* strains.^{11,13} Recognized information revealed that *H. pylori* bacteria displayed the boost incidence of resistance toward diverse kinds of antibiotics such as tetracyclines, fluoroquinolone, aminoglycosides, penicillins, sulfonamides, and macrolides.^{11,13} The presence of certain antibiotic resistance genes, particularly *rdxA*, *pbp1A*, *gyrA*, and *cla* which encode resistance toward metronidazole, amoxicillin, fluoroquinolone, and clarithromycin antibiotic agents, respectively, is one of the most important reasons for occurrence of antibiotic resistance.^{14,15} Therefore, it is significant to know the exact phenotypic and genotypic patterns of antibiotic resistance of *H. pylori* bacteria recovered from foods with animal origins.

Data on the epidemiology and transmission of *H. pylori* is extremely significant in order to prevent its distribution and to identify high-risk populations. Considering the indistinct epidemiological aspects of *H. pylori* in meat, as a highly consumed foodstuff, an existing research was performed in order to assess the incidence, genotyping patterns and phenotypic and genotypic profiles of antibiotic resistance of the *H. pylori* bacteria recovered from raw meat samples of camel, caprine, ovine, bovine, and buffalo species.

Materials and Methods

Samples

From April to October 2018, a total 600 raw meat samples including bovine (n= 140), ovine (n=130), caprine (n= 130), buffalo (n= 100), and camel (n= 100) were arbitrarily collected from the butchers of diverse areas of Tehran province, Iran. All meat samples were collected from the femur muscle. Meat samples displayed natural physical (color, odor, pH, and density) constancy. Samples (40 g, in sterile glass bottles) were transported in ice-cooled flasks (at 4°C) to the laboratory within 2 hours after collection.

Isolation of *Helicobacter pylori*

Isolation of *H. pylori* bacteria was performed using the culture technique.^{17,18} Twenty-five grams of meat sample were applied for this resolve. Wilkins Chalgren anaerobe broth (Oxoid Ltd., Basingstoke, UK) was applied for this goal. Culture media were supplemented with 5% of horse serum (Sigma, St. Louis, MO), nalidixic acid (30 mg/L), vancomycin

(10 mg/L), cycloheximide (100 mg/L), and trimethoprim (30 mg/L) (Sigma). Microaerophilic circumstances (5% oxygen, 85% nitrogen, and 10% CO₂) was equipped using the MART system (MART system, Lichtenvoorde, The Netherlands). For comparison, a reference strain of *H. pylori* (ATCC 43504) was employed. Suspected colonies were then identified using colony morphology, Gram staining, and some biochemical tests such as urease, oxidase, catalase, and nitrate reduction.

DNA Extraction and 16S rRNA-Based Polymerase Chain Reaction (PCR) Confirmation

H. pylori isolates were additionally confirmed using the 16S rRNA-based PCR method. Colonies were sub-cultured on Wilkins Chalgren anaerobe broth supplemented with the same materials declared above.^{16,17} Genomic DNA was then extracted using a DNA extraction kit (Thermo Fisher Scientific, St. Leon-Rot, Germany). Technique was performed rendering to the factory guidelines. Purity (A260/A280) and concentration of extracted DNA were then plaid (NanoDrop, Thermo Scientific, Waltham, MA) and the DNA quality was scrutinized by electrophoresis. PCR was accompanied using a PCR thermal cycler (Eppendorf Co., Hamburg, Germany) rendering to the described procedure.¹⁸ *H. pylori* 26695 was used as positive, while sterile PCR grade water (Thermo Fisher Scientific) was used as negative controls.

Study the Antibiotic Resistance Pattern

Mueller–Hinton agar (Merck, Germany) was applied to assess the pattern of antibiotic resistance using the simple disk diffusion technique. Antibiotic resistance profile of *H. pylori* bacteria was researched toward dissimilar antibiotic agents (Oxoid, UK) using the guidelines of the Clinical and Laboratory Standards Institute (CLSI).^{19,20} Resistance patterns of bacteria were experienced toward levofloxacin (5 µg), ampicillin (10 µg), clarithromycin (2 µg), metronidazole (5 µg), streptomycin (10 µg), amoxicillin (10 µg), cefsulodin (30 µg), tetracycline (30 µg), erythromycin (5 µg), furazolidone (1 µg), trimethoprim (25 µg), rifampin (30 µg), and spiramycin (100 µg) (Oxoid). Positive controls (NCTC 13206 (CCUG 38770) and NCTC 13207 (CCUG 38772)) were accompanied in this experiment.

Study the Distribution of Antibiotic Resistance Genes and Genotyping Pattern

Distribution of antibiotic resistance genes and *vacA*, *cagA*, *iceA*, *oipA*, *cagE*, and *babA2* genotypes of *H. pylori* bacteria were assessed rendering the preceding experiment.^{21,29} PCR circumstances were displayed in Table 1. Positive (SS1 (for *cagA* and *cagE* genotypes), 26,695 (for *babA2*, *vacA*, *cagA*, *cagE*, *iceA* genotypes and *cla* and *rdxA* antibiotic resistance genes), Tx30 (for *vacA* genotypes), J99 (for *cagA* and *babA2*), 88–23 (for *cagA* and *vacA* genotypes), 84–183 (for *vacA* and *cagA* genotypes), 43,504 (for *vacA* and *iceA2* genotypes), 49,503 (for *iceA1* genotypes), D0008 (for *oipA* genotype), 69A (for *rdxA* and *pbp 1A* antibiotic resistance gene), and RM92 (for *gyrA* antibiotic resistance gene), and negative (PCR grade water (Thermo Fisher Scientific)) controls were also accompanied in this experiment. Electrophoresis was addressed rendering previous experiments.²¹

Numerical Examination

Data were subjected to Microsoft office Excel (version 15; Microsoft Corp., Redmond, WA). Numerical examination was performed by means of the SPSS 21.0 numerical software (SPSS Inc., Chicago, IL). Chi-square test and Fisher's exact two-tailed test were applied to measure any momentous relationship. *P*-value<0.05 was considered as a numerical momentous level.

Results

Table 2 embodies the incidence of *H. pylori* bacteria recovered from diverse kinds of raw meat samples. Fifty-two out of 600 (8.66%) raw meat samples were contaminated with *H. pylori*. Raw ovine (13.07%) samples had the uppermost contamination rate with *H. pylori* bacteria, while raw camel (3%) had the lowest. Numerical momentous variance was originated amid kinds of samples and incidence of *H. pylori* bacteria (*P*<0.05).

Table 3 embodies the antibiotic resistance pattern of *H. pylori* bacteria recovered from diverse kinds of raw meat samples. *H. pylori* bacteria displayed the uppermost incidence of resistance toward tetracycline (82.69%), erythromycin (80.76%), trimethoprim (65.38%), levofloxacin (63.46%), amoxicillin (63.46%), and clarithromycin (61.53%) antibiotic agents. *H. pylori* bacteria displayed the lowest incidence of resistance toward spiramycin (21.15%), furazolidone (25%), cefsulodin (38.46%), and rifampin (40.38%) antibiotic agents. *H. pylori* bacteria recovered

Table 1 Set of Primers and PCR Circumstances Applied for Detection of Antibiotic Resistance Genes and Genotyping of *vacA*, *cagA*, *iceA*, *oipA*, *cagE*, and *babA2* Alleles

Genes		Primer Sequence (5'-3')	Size of Product (Bp)	Volume of PCR Reaction (50 µl)	PCR Programs
<i>VacA</i> <i>s</i> _{1a}		F: CTCTCGCTTTAGTAGGAGC R: CTGCTTGAATGCGCCAAAC	213	5 µL PCR buffer 10X 1.5 mM MgCl ₂ 200 µM dNTP (Thermo Fisher Scientific) 0.5 µM of each primers F & R 1.25 U Taq DNA polymerase (Thermo Fisher Scientific) 2.5 µL DNA template	1 cycle: 95°C; 1 min. 32 cycle: 95°C; 45 s
<i>VacA</i> <i>s</i> _{1b}		F: AGCGCCATACCGCAAGAG R: CTGCTTGAATGCGCCAAAC	187		64°C; 50 s 72°C; 70 s
<i>VacA</i> <i>s</i> _{1c}		F: CTCTCGCTTTAGTGGGGYT R: CTGCTTGAATGCGCCAAAC	213		1 cycle: 72°C; 5 min
<i>VacA</i> <i>s</i> ₂		F: GCTAACACGCCAAATGATCC R: CTGCTTGAATGCGCCAAAC	199		
<i>VacA</i> <i>m</i> _{1a}		F: GGTCAAATGCGGTCATGG R: CCATTGGTACCTGTAGAAAC	290		
<i>VacA</i> <i>m</i> _{1b}		F: GGCCCAATGCAGTCATGGA R: GCTGTTAGTGCCTAAAGAAGCAT	291		
<i>VacA</i> <i>m</i> ₂		F: GGAGCCCCAGAAACATTG R: CATAACTAGCGCCTTGCA	352		
<i>Cag A</i>		F: GATAACAGCCAAGCTTTTGAGG R: CTGCAAAAGATTGTTGGCAGA	300	5 µL PCR buffer 10X 2 mM MgCl ₂ 150 µM dNTP (Thermo Fisher Scientific) 0.75 µM of each primers F & R 1.5 U Taq DNA polymerase (Thermo Fisher Scientific) 3 µL DNA template	1 cycle: 94°C; 1 min. 32 cycle: 95°C; 60 s 56°C; 60 s 72°C; 60 s 1 cycle: 72°C; 10 min
<i>IceA</i>	<i>iceA1</i>	F: GTGTTTTTAACCAAAGTATC R: CTATAGCCASTYTCTTTGCA	247	5 µL PCR buffer 10X 2 mM MgCl ₂ 150 µM dNTP (Thermo Fisher Scientific) 0.75 µM of each primers F & R 1.5 U Taq DNA polymerase (Thermo Fisher Scientific) 3 µL DNA template	1 cycle: 94°C; 1 min. 32 cycle: 94°C; 60 s 56°C; 60 s 72°C; 60 s 1 cycle: 72°C; 10 min
	<i>iceA2</i>	F: GTTGGGTATATCACAATTTAT R: TTRCCCTATTTCTAGTAGGT	229/334		
<i>OipA</i>		F: GTTTTTGATGCATGGGATTT R: GTGCATCTCTTATGGCTTT	401	5 µL PCR buffer 10X 2 mM MgCl ₂ 150 µM dNTP (Thermo Fisher Scientific) 0.75 µM of each primers F & R 1.5 U Taq DNA polymerase (Thermo Fisher Scientific) 3 µL DNA template	1 cycle: 94°C; 1 min. 32 cycle: 94°C; 60 s 56°C; 60 s 72°C; 60 s 1 cycle: 72°C; 10 min
<i>cagE</i>		F: TTGAAACTTCAAGGATAGGATAGAGC R: GCCTAGCGTAATATCACCATTAACC	500	5 µL PCR buffer 10X 2 mM MgCl ₂ 150 µM dNTP (Thermo Fisher Scientific) 0.75 µM of each primers F & R 1.5 U Taq DNA polymerase (Thermo Fisher Scientific) 3 µL DNA template	1 cycle: 95°C; 4 min. 31 cycle: 95°C; 44 s 51°C; 45 s 72°C; 62 s 1 cycle: 72°C; 5 min

(Continued)

Table 1 (Continued).

Genes		Primer Sequence (5'-3')	Size of Product (Bp)	Volume of PCR Reaction (50 µl)	PCR Programs
<i>BabA2</i>		F: CCAAACGAAACAAAAGCGT R: GCTTGTGTA AAAAGCCGTCGT	105–124	5 µL PCR buffer 10X 2 mM MgCl ₂ 150 µM dNTP (Thermo Fisher Scientific) 0.75 µM of each primers F & R 1.5 U Taq DNA polymerase (Thermo Fisher Scientific) 3 µL DNA template	1 cycle: 94°C; 1 min. 35 cycle: 94°C; 60 s 57°C; 45 s 72°C; 30 s 1 cycle: 72°C; 10 min
<i>rdxA</i>	Metronidazole	F: AATTTGAGCATGGGGCAGA R: GAAACGCTTGAAAACCCCT	581	5 µL PCR buffer 10X 2 mM MgCl ₂ 150 µM dNTP (Thermo Fisher Scientific) 0.75 µM of each primers F & R 1.5 U Taq DNA polymerase (Thermo Fisher Scientific) 3 µL DNA template	1 cycle: 95°C; 5 min. 40 cycle: 94°C; 30 s 55°C; 30 s 72°C; 60 s 1 cycle: 72°C; 10 min
<i>pbp1A</i>	Amoxicillin	F: GCGACAATAAGAGTGGCA R: TGCGAACACCCTTTTAA T	2,300	5 µL PCR buffer 10X 2 mM MgCl ₂ 150 µM dNTP (Thermo Fisher Scientific) 0.75 µM of each primers F & R 1.5 U Taq DNA polymerase (Thermo Fisher Scientific) 3 µL DNA template	1 cycle: 95°C; 3 min. 35 cycle: 95°C; 60 s 54°C; 60 s 72°C; 5 min 1 cycle: 72°C; 10 min
<i>gyrA</i>	Fluoroquinolone	F: TTTTGCTTATTCMATGAGCGT R: GCAGACGGCTTGGTARAATA	2,300	5 µL PCR buffer 10X 2 mM MgCl ₂ 150 µM dNTP (Thermo Fisher Scientific) 0.75 µM of each primers F & R 1.5 U Taq DNA polymerase (Thermo Fisher Scientific) 3 µL DNA template	1 cycle: 94°C; 5 min. 35 cycle: 94°C; 30 s 47°C; 30 s 72°C; 30 s 1 cycle: 72°C; 5 min
<i>cla</i>	Clarithromycin	F: AGTCGGGACCTAAGGCGAG R: AGGTCCACCACGGGGTCTTG	700	5 µL PCR buffer 10X 2 mM MgCl ₂ 150 µM dNTP (Thermo Fisher Scientific) 0.75 µM of each primers F & R 1.5 U Taq DNA polymerase (Thermo Fisher Scientific) 3 µL DNA template	1 cycle: 94°C; 5 min. 30 cycle: 94°C; 60 s 55°C; 60 s 72°C; 60 s 1 cycle: 72°C; 5 min

from raw ovine meat samples displayed the most diverse incidence of resistance toward antibiotic agents. Numerical momentous variance was originated amid kinds of samples and incidence of antibiotic resistance of *H. pylori* bacteria ($P<0.05$). Figure 1 embodies the distribution of multi-drug resistant *H. pylori* bacteria recovered from diverse kinds of raw meat samples. All *H. pylori* bacteria recovered from raw meat samples had at least resistance toward one antibiotic

agent, while incidence of resistance toward more than eight types of antibiotics was 28.84%.

Table 4 embodies the distribution of antibiotic resistance genes amongst the *H. pylori* bacteria recovered from diverse kinds of raw meat samples. Total distribution of *rdxA*, *pbp1A*, *gyrA*, and *cla* antibiotic resistance genes amongst the *H. pylori* bacteria recovered from diverse kinds of raw meat samples were 59.61%, 51.92%,

Table 2 Incidence of *H. pylori* in Diverse Kinds of Raw Meat Samples

Raw Milk Samples	No Samples Collected	N (%) of <i>H. pylori</i> Positive Samples*
Bovine	140	8 (5.71)
Ovine	130	17 (13.07)
Caprine	130	15 (11.53)
Buffalo	100	9 (9)
Camel	100	3 (3)
Total	600	52 (8.66)

Note: **H. pylori* isolates were also confirmed by the 16S rRNA-based PCR amplification.

69.23%, and 65.38%, respectively. *H. pylori* bacteria recovered from raw ovine meat samples displayed the most diverse distribution of antibiotic resistance genes. Numerical momentous variance was originated amid kinds of samples and distribution of antibiotic resistance genes ($P<0.05$).

Table 5 embodies the distribution of alleles amongst the *H. pylori* bacteria recovered from diverse kinds of raw meat samples. *vacA s1a* (84.61%), *s2* (76.92%), *m1a* (50%), *m2* (39.13%), *iceA1* (38.46%), and *cagA* (55.76%) were the most generally perceived alleles amongst the *H. pylori* bacteria. Distribution of *vacA s1c* (7.69%) and *m1b* (21.15%) and *iceA2* (7.69%) and *babA2* (19.23%) alleles were lower than other detected genotypes. *H. pylori* bacteria recovered from raw ovine meat samples displayed the most diverse distribution of alleles. Numerical momentous variance was originated between type of samples and distribution of alleles of *H. pylori* bacteria ($P<0.05$). Furthermore, numerical momentous variance was originated amid distribution of *cagA* and *cagE* ($P<0.01$) and *iceA1* and *iceA2* ($P<0.01$) alleles.

Table 6 embodies the genotyping pattern of *H. pylori* bacteria recovered from diverse kinds of raw meat samples. *S1am1a* (63.46%), *s2m1a* (53.84%), *s1am2* (51.92%), and *s2m2* (42.30%) were the most generally perceived genotyping pattern of the *vacA* alleles of *H. pylori* bacteria recovered from diverse kinds of raw meat samples. Distribution of *cagA*-, *oipA*-, and *babA2*-genotypes were 44.23%, 73.07%, and 80.76%, respectively. We originated that 5.76% of *H. pylori* bacteria displayed *iceA1/iceA2* genotyping pattern. *S1cm1b* (1.92%), *s1cm2* (3.84%), *s1cm1a* (3.84%), and *s1bm1b* (7.62%) had the lowest incidence amongst different genotyping patterns of *H. pylori* bacteria. *H. pylori* bacteria recovered from raw ovine meat samples displayed the most diverse distribution of genotypes.

Table 3 Antibiotic Resistance Pattern of *H. pylori* Bacteria Isolated from Diverse Kinds of Raw Meat Samples

Type of Raw Meat Samples (N H. Pylori Bacteria)	N (%) Isolates Resistant to Each Antibiotic												
	Am10	Clr2	Er5	Met5	Lev5	Tet30	AMX 10	Rif30	Trp25	Cef30	S10	Fz11	Spil100
Bovine (8)	5 (62.50)	4 (50)	6 (75)	3 (37.50)	4 (50)	7 (87.50)	4 (50)	3 (37.50)	4 (50)	3 (37.50)	4 (50)	2 (25)	2 (25)
Ovine (17)	15 (88.23)	12 (70.58)	15 (88.23)	9 (52.94)	12 (70.58)	15 (88.23)	12 (70.58)	7 (41.17)	13 (76.47)	6 (35.29)	11 (64.70)	5 (29.41)	4 (23.52)
Caprine (15)	12 (80)	10 (66.66)	12 (80)	10 (66.66)	11 (73.33)	12 (80)	10 (66.66)	6 (40)	11 (73.33)	6 (40)	9 (60)	3 (20)	3 (20)
Buffalo (9)	6 (66.66)	5 (55.55)	7 (77.77)	4 (44.44)	5 (55.55)	7 (77.77)	5 (55.55)	4 (44.44)	5 (55.55)	4 (44.44)	5 (55.55)	2 (22.22)	2 (22.22)
Camel (3)	1 (33.33)	1 (33.33)	2 (66.66)	1 (33.33)	1 (33.33)	2 (66.66)	2 (66.66)	1 (33.33)	1 (33.33)	1 (33.33)	2 (66.66)	1 (33.33)	-
Total (52)	31 (59.61)	32 (61.53)	42 (80.76)	27 (51.92)	33 (63.46)	43 (82.69)	33 (63.46)	21 (40.38)	34 (65.38)	20 (38.46)	31 (59.61)	13 (25)	11 (21.15)

Abbreviations: AM10, ampicillin (10 µg); CLR2, clarithromycin (2 µg); ER5, erythromycin (5 µg); Met5, metronidazole (5 µg); Lev5, levofloxacin (5 µg); Tet30, tetracycline (30 µg); AMX10, amoxicillin (10 µg); Rif30, rifampin (30 µg); TRP25, trimethoprim (25 µg); Cef30, cefsulodin (30 µg); S10, streptomycin (10 µg); FZL1, furazolidone (1 µg); Spil100, spiramycin (100 µg).

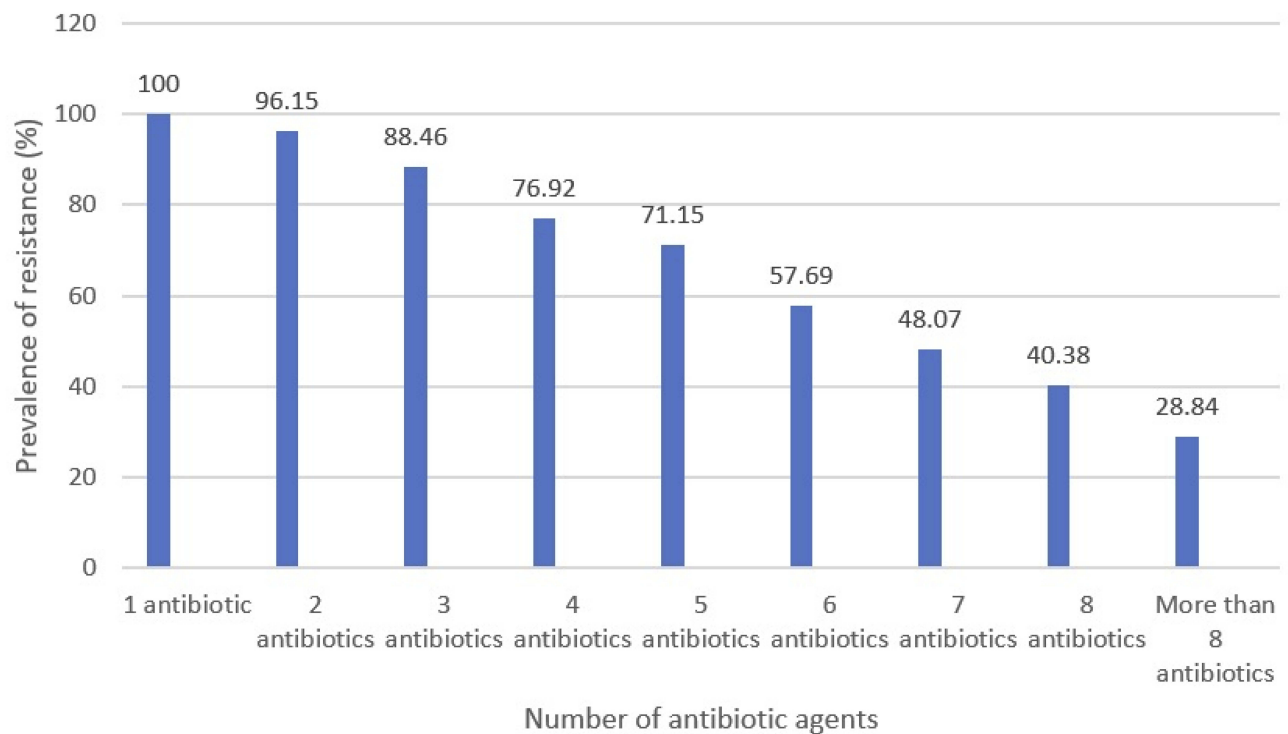


Figure 1 Incidence of multi-drug resistant *H. pylori* bacteria isolated from raw meat samples.

Table 7 embodies the combined genotyping pattern of *H. pylori* bacteria recovered from diverse kinds of raw meat samples. We originated that s1a/cagA-/iceA1/oipA-/cagE-/babA- (32.69%), m1a/cagA-/iceA1/oipA-/cagE-/babA- (32.69%), s2/cagA-/iceA1/oipA-/cagE-/babA- (26.92%), s1a/cagA+/iceA1/oipA+/cagE-/babA- (23.07%), m1a/cagA+/iceA1/oipA+/cagE+/babA- (23.07%), m1a/cagA+/iceA1/oipA+/cagE-/babA- (23.07%), m2/cagA-/iceA1/oipA-/cagE-/babA- (23.07%), s1a/cagA+/iceA1/oipA+/cagE+/babA- (21.15%), s1a/cagA-/iceA1/oipA-/cagE+/babA- (21.15%), s1a/cagA-/iceA1/oipA+/cagE-/babA- (21.15%), s1b/cagA-/iceA1/oipA-/cagE-/babA- (21.15%), m1a/cagA-/iceA1/oipA-/cagE+/babA- (21.15%), m1a/cagA-/iceA1/oipA+/cagE-/babA- (21.15%),

gE-/babA- (21.15%), m1b/cagA-/iceA1/oipA-/cagE-/babA- (21.15%), s1a/cagA+/iceA1/oipA-/cagE+/babA- (19.23%), s2/cagA+/iceA1/oipA+/cagE-/babA- (19.23%), s2/cagA-/iceA1/oipA-/cagE+/babA- (19.23%), s2/cagA-/iceA1/oipA+/cagE-/babA- (19.23%), m1a/cagA+/iceA1/oipA-/cagE-/babA+ (19.23%), and m1a/cagA+/iceA1/oipA-/cagE+/babA- (19.23%) were the most generally perceived combined genotyping pattern of *H. pylori* bacteria recovered from diverse kinds of raw meat samples. Incidence of s1a/cagA+/iceA2/oipA+/cagE+/babA+, s1a/cagA+/iceA2/oipA+/cagE-/babA+, s1a/cagA-/iceA2/oipA+/cagE+/babA+, s1a/cagA+/iceA2/oipA-/cagE+/babA-, s1a/cagA-/iceA2/oipA+/cagE-/babA-, s1b/cagA+/iceA1/oipA

Table 4 Distribution of Antibiotic Resistant Genes Amongst the *H. pylori* Bacteria Isolated from Diverse Kinds of Raw Meat Samples

Type of Raw Meat Samples (N <i>H. pylori</i> Bacteria)	N (%) Isolates Harbored Each Antibiotic Resistant Gene			
	Metronidazole	Amoxicillin	Fluoroquinolone	Clarithromycin
	<i>rdxA</i>	<i>pbp1A</i>	<i>gyrA</i>	<i>cla</i>
Bovine (8)	4 (50)	5 (62.50)	4 (50)	5 (62.50)
Ovine (17)	11 (64.70)	13 (76.47)	13 (76.47)	12 (70.58)
Caprine (15)	10 (66.66)	12 (80)	12 (80)	11 (73.33)
Buffalo (9)	5 (55.55)	6 (66.66)	5 (55.55)	5 (55.55)
Camel (3)	1 (33.33)	1 (33.33)	2 (66.66)	1 (33.33)
Total (52)	31 (59.61)	27 (51.92)	36 (69.23)	34 (65.38)

Table 5 Distribution of Alleles Amongst the *H. pylori* Bacteria Isolated from Diverse Kinds of Raw Meat Samples

Type of Raw Meat Samples (N <i>H. pylori</i> Bacteria)	N (%) Isolates Harbor Each Genotype														
	VacA						CagA			IceA		OipA		CagE	BabA2
	s/a	s/b	s/c	s2	m/a	m/b	m2	m1a	m1b	m2	IceA1	IceA2	OipA	CagE	BabA2
Bovine (8)	6 (75)	1 (12.50)	-	6 (75)	7 (87.50)	1 (12.50)	6 (75)	7 (87.50)	1 (12.50)	4 (50)	1 (12.50)	2 (25)	3 (37.50)	2 (25)	
Ovine (17)	16 (94.11)	6 (35.29)	2 (11.76)	15 (88.23)	16 (94.11)	5 (29.41)	14 (82.35)	16 (94.11)	7 (41.17)	7 (41.17)	2 (11.76)	5 (29.41)	4 (23.52)	3 (17.64)	
Caprine (15)	13 (86.66)	4 (26.66)	1 (6.66)	12 (80)	13 (86.66)	3 (20)	10 (66.66)	13 (86.66)	5 (33.33)	5 (33.33)	1 (6.66)	4 (26.66)	3 (20)	3 (20)	
Buffalo (9)	6 (66.66)	2 (22.22)	1 (11.11)	7 (77.77)	8 (88.88)	2 (22.22)	5 (55.55)	8 (88.88)	3 (33.33)	3 (33.33)	-	2 (22.22)	2 (22.22)	2 (22.22)	
Camel (3)	3 (100)	-	-	2 (66.66)	2 (66.66)	-	1 (33.33)	2 (66.66)	1 (33.33)	1 (33.33)	-	1 (33.33)	1 (33.33)	-	
Total (52)	44 (84.61)	13 (25)	4 (7.69)	40 (76.92)	46 (50)	11 (21.15)	36 (39.13)	46 (50)	29 (55.76)	20 (38.46)	4 (7.69)	14 (26.92)	13 (25)	10 (19.23)	

+/cagE+/babA+, s1b/cagA-/iceA1/oipA-/cagE+/babA+, s1c/cagA+/iceA1/oipA+/cagE+/babA-, s1c/cagA+/iceA1/oipA+/cagE-/babA-, s1c/cagA-/iceA1/oipA-/cagE+/babA-, s2/cagA+/iceA2/oipA+/cagE+/babA-, s2/cagA+/iceA2/oipA+/cagE-/babA-, s2/cagA-/iceA2/oipA-/cagE-/babA-, m1a/cagA+/iceA2/oipA+/cagE+/babA+, m1a/cagA+/iceA2/oipA+/cagE-/babA+, m1a/cagA-/iceA2/oipA-/cagE+/babA-, m1b/cagA-/iceA1/oipA-/cagE+/babA+, m1b/cagA+/iceA2/oipA+/cagE-/babA-, m1b/cagA-/iceA2/oipA-/cagE-/babA-, m2/cagA+/iceA2/oipA+/cagE+/babA-, m2/cagA+/iceA2/oipA+/cagE-/babA-, and m2/cagA-/iceA2/oipA-/cagE-/babA- (1.92%) were lower than other detected combined genotyping patterns.

Discussion

H. pylori is a common bacterium with considerable clinical rank. About 50% of the world’s population have been infected with *H. pylori* bacteria. Despite the boost occurrence of infection, the main reservoir of the bacterium and the routes of infections are still unspecified.³⁰ Furthermore, bacterial transmission between persons ensues through the oral–oral and oral–fecal routes.³⁰ However, oral–fecal transmission has additional implications, since *H. pylori* may occur in food and water supplies subsequent to fecal contamination.³⁰ Additionally, isolation of *H. pylori* from raw vegetables,^{16,31} meat,^{32,33} salads,^{16,34} ready to eat foods,^{35,36} and milk^{37,38} proposes that foodstuffs may act as vehicles for transmission of *H. pylori* to human community.

The current survey was carried out in order to assess the incidence, phenotypic and genotypic pattern of antibiotic resistance and genotyping profile of *vacA*, *cagA*, *cagE*, *iceA*, *oipA*, and *babA* alleles of the *H. pylori* bacteria recovered from raw camel, caprine, ovine, bovine, and buffalo meat samples. The contamination rate of *H. pylori* in bovine, ovine, caprine, buffalo, and camel meat samples was 5.71%, 13.07%, 11.53%, 9%, and 3%, respectively. Despite the higher importance of meat as a food which is served as so many kinds of undercooked products and therefore its higher risk of contamination with *H. pylori*, scarce data are available in this field. Saedi and Sheikhshahrokh³⁹ stated that the incidence of *H. pylori* bacteria amongst the raw cow, sheep, goat, buffalo, and camel meat samples were 25%, 37%, 22%, 28%, and 14%, respectively. Gilani et al⁴⁰ stated that the incidence of *H. pylori* bacteria amongst the hamburger and minced meat samples were 1.42% and 12.50%, respectively. Additionally, *H. pylori* DNA was detected in 44% and 36% of ready-to-eat raw tuna meat and raw chicken

Table 6 Genotyping Pattern of *H. pylori* Bacteria Isolated from Diverse Kinds of Raw Meat Samples

Type of Raw Meat Samples (N of <i>H. pylori</i> Bacteria)	Genotyping pattern (%)																					
	<i>slam1a</i>	<i>slam1b</i>	<i>slam2</i>	<i>slbm1a</i>	<i>slbm1b</i>	<i>slbm2</i>	<i>slcm1a</i>	<i>slcm1b</i>	<i>slcm2</i>	<i>szm1a</i>	<i>szm1b</i>	<i>szm2</i>	<i>CagA+</i>	<i>CagA-</i>	<i>IceA1/IceA2</i>	<i>OipA+</i>	<i>OipA-</i>	<i>CagE+</i>	<i>CagE-</i>	<i>BabA2+</i>	<i>BabA2-</i>	
Bovine (8)	4 (50)	1 (12.50)	4 (50)	1 (12.50)	-	-	-	-	-	3 (37.50)	1 (12.50)	3 (37.50)	5 (62.50)	3 (37.50)	1 (12.50)	2 (25)	6 (75)	3 (37.50)	5 (62.50)	2 (25)	6 (75)	
Ovine (17)	12 (70.58)	3 (17.64)	11 (64.70)	2 (11.76)	2 (11.76)	3 (17.64)	1 (5.88)	1 (5.88)	1 (5.88)	11 (64.70)	2 (11.76)	10 (58.82)	12 (70.58)	5 (29.41)	1 (5.88)	5 (29.41)	12 (70.58)	4 (23.52)	13 (76.47)	3 (17.64)	14 (82.35)	
Caprine (15)	11 (73.33)	2 (13.33)	8 (53.33)	2 (13.33)	1 (6.66)	2 (13.33)	-	-	1 (6.66)	8 (53.33)	1 (6.66)	9 (60)	8 (53.33)	7 (46.66)	1 (6.66)	4 (26.66)	11 (73.33)	3 (20)	12 (80)	3 (20)	12 (80)	
Buffalo (9)	5 (55.55)	1 (11.11)	3 (21.42)	1 (11.11)	1 (11.11)	1 (11.11)	-	-	-	5 (55.55)	1 (11.11)	4 (44.44)	3 (33.33)	6 (66.66)	-	2 (22.22)	7 (77.77)	2 (22.22)	7 (77.77)	2 (22.22)	7 (77.77)	
Camel (3)	1 (33.33)	-	1 (33.33)	-	-	-	-	-	-	1 (33.33)	-	1 (33.33)	1 (33.33)	2 (66.66)	-	1 (33.33)	2 (66.66)	1 (33.33)	2 (66.66)	-	3 (100)	
Total (52)	33 (63.46)	7 (13.46)	27 (51.92)	7 (13.46)	4 (7.69)	6 (11.53)	2 (3.84)	1 (1.92)	2 (3.84)	28 (53.84)	5 (9.61)	22 (42.30)	29 (55.76)	23 (44.23)	3 (5.76)	14 (26.92)	38 (73.07)	13 (25)	39 (75)	10 (19.23)	42 (80.76)	

Table 7 Combined Genotyping Pattern of *H. pylori* Bacteria Isolated from Diverse Kinds of Raw Meat Samples

Combined Genotyping Patterns	Distribution* (%)
Sl a/cagA+/iceA1/oipA+/cagE+/babA+	6 (11.53)
Sl a/cagA+/iceA1/oipA+/cagE+/babA-	11 (21.15)
Sl a/cagA+/iceA1/oipA+/cagE-/babA+	8 (15.38)
Sl a/cagA+/iceA1/oipA-/cagE+/babA+	7 (13.46)
Sl a/cagA-/iceA1/oipA+/cagE+/babA+	8 (15.38)
Sl a/cagA+/iceA1/oipA+/cagE-/babA-	12 (23.07)
Sl a/cagA+/iceA1/oipA-/cagE-/babA+	9 (17.30)
Sl a/cagA+/iceA1/oipA-/cagE+/babA-	10 (19.23)
Sl a/cagA-/iceA1/oipA-/cagE+/babA-	11 (21.15)
Sl a/cagA-/iceA1/oipA-/cagE+/babA+	7 (13.46)
Sl a/cagA-/iceA1/oipA+/cagE-/babA+	6 (11.53)
Sl a/cagA-/iceA1/oipA+/cagE+/babA-	9 (17.30)
Sl a/cagA-/iceA1/oipA+/cagE-/babA-	11 (21.15)
Sl a/cagA-/iceA1/oipA-/cagE-/babA-	17 (32.69)
Sl a/cagA+/iceA2/oipA+/cagE+/babA+	1 (1.92)
Sl a/cagA+/iceA2/oipA+/cagE+/babA-	2 (3.84)
Sl a/cagA+/iceA2/oipA+/cagE-/babA+	1 (1.92)
Sl a/cagA+/iceA2/oipA-/cagE+/babA+	-
Sl a/cagA-/iceA2/oipA+/cagE+/babA+	1 (1.92)
Sl a/cagA+/iceA2/oipA+/cagE-/babA-	2 (3.84)
Sl a/cagA+/iceA2/oipA-/cagE-/babA+	-
Sl a/cagA+/iceA2/oipA-/cagE+/babA-	1 (1.92)
Sl a/cagA-/iceA2/oipA+/cagE+/babA+	-
Sl a/cagA-/iceA2/oipA-/cagE+/babA+	-
Sl a/cagA-/iceA2/oipA+/cagE-/babA+	-
Sl a/cagA-/iceA2/oipA+/cagE+/babA-	1 (1.92)
Sl a/cagA-/iceA2/oipA+/cagE-/babA-	1 (1.92)
Sl a/cagA-/iceA2/oipA-/cagE-/babA-	3 (5.76)
Sl b/cagA+/iceA1/oipA+/cagE+/babA+	1 (1.92)
Sl b/cagA+/iceA1/oipA+/cagE+/babA-	5 (9.61)
Sl b/cagA+/iceA1/oipA+/cagE-/babA+	2 (3.84)
Sl b/cagA+/iceA1/oipA-/cagE+/babA+	2 (3.84)
Sl b/cagA-/iceA1/oipA+/cagE+/babA+	2 (3.84)
Sl b/cagA+/iceA1/oipA+/cagE-/babA-	6 (11.53)
Sl b/cagA+/iceA1/oipA-/cagE-/babA+	3 (5.76)
Sl b/cagA+/iceA1/oipA-/cagE+/babA-	4 (7.69)
Sl b/cagA-/iceA1/oipA-/cagE+/babA-	5 (9.61)
Sl b/cagA-/iceA1/oipA-/cagE+/babA+	1 (1.92)
Sl b/cagA-/iceA1/oipA+/cagE-/babA+	-
Sl b/cagA-/iceA1/oipA+/cagE+/babA-	3 (5.76)
Sl b/cagA-/iceA1/oipA+/cagE-/babA-	5 (9.61)
Sl b/cagA-/iceA1/oipA-/cagE-/babA-	11 (21.15)
Sl b/cagA+/iceA2/oipA+/cagE+/babA+	-
Sl b/cagA+/iceA2/oipA+/cagE+/babA-	-
Sl b/cagA+/iceA2/oipA+/cagE-/babA+	-
Sl b/cagA+/iceA2/oipA-/cagE+/babA+	-
Sl b/cagA-/iceA2/oipA+/cagE+/babA+	-
Sl b/cagA+/iceA2/oipA+/cagE-/babA-	-
Sl b/cagA+/iceA2/oipA-/cagE-/babA+	-
Sl b/cagA+/iceA2/oipA-/cagE+/babA-	-

(Continued)

Table 7 (Continued).

Combined Genotyping Patterns	Distribution* (%)
Sl b/cagA-/iceA2/oipA+/cagE+/babA+	-
Sl b/cagA-/iceA2/oipA-/cagE+/babA+	-
Sl b/cagA-/iceA2/oipA+/cagE-/babA+	-
Sl b/cagA-/iceA2/oipA+/cagE+/babA-	-
Sl b/cagA-/iceA2/oipA+/cagE-/babA-	-
Sl b/cagA-/iceA2/oipA-/cagE-/babA-	-
Sl c/cagA+/iceA1/oipA+/cagE+/babA+	-
Sl c/cagA+/iceA1/oipA+/cagE+/babA-	1 (1.92)
Sl c/cagA+/iceA1/oipA+/cagE-/babA+	-
Sl c/cagA+/iceA1/oipA-/cagE+/babA+	-
Sl c/cagA-/iceA1/oipA+/cagE+/babA+	-
Sl c/cagA+/iceA1/oipA+/cagE-/babA-	1 (1.92)
Sl c/cagA+/iceA1/oipA-/cagE-/babA+	-
Sl c/cagA+/iceA1/oipA-/cagE+/babA-	-
Sl c/cagA-/iceA1/oipA-/cagE+/babA-	1 (1.92)
Sl c/cagA-/iceA1/oipA-/cagE+/babA+	-
Sl c/cagA-/iceA1/oipA+/cagE-/babA+	-
Sl c/cagA-/iceA1/oipA+/cagE+/babA-	-
Sl c/cagA-/iceA1/oipA+/cagE-/babA-	-
Sl c/cagA-/iceA1/oipA-/cagE-/babA-	2 (3.84)
Sl c/cagA+/iceA2/oipA+/cagE+/babA+	-
Sl c/cagA+/iceA2/oipA+/cagE+/babA-	-
Sl c/cagA+/iceA2/oipA+/cagE-/babA+	-
Sl c/cagA+/iceA2/oipA-/cagE+/babA+	-
Sl c/cagA-/iceA2/oipA+/cagE+/babA+	-
Sl c/cagA+/iceA2/oipA+/cagE-/babA-	-
Sl c/cagA+/iceA2/oipA-/cagE-/babA+	-
Sl c/cagA+/iceA2/oipA-/cagE+/babA-	-
Sl c/cagA-/iceA2/oipA+/cagE+/babA+	-
Sl c/cagA-/iceA2/oipA-/cagE+/babA+	-
Sl c/cagA-/iceA2/oipA+/cagE+/babA-	-
Sl c/cagA-/iceA2/oipA+/cagE-/babA+	-
Sl c/cagA-/iceA2/oipA-/cagE+/babA-	-
Sl c/cagA-/iceA2/oipA-/cagE+/babA+	-
Sl c/cagA-/iceA2/oipA+/cagE+/babA-	-
Sl c/cagA-/iceA2/oipA+/cagE-/babA+	-
Sl c/cagA-/iceA2/oipA-/cagE+/babA-	-
Sl c/cagA-/iceA2/oipA-/cagE-/babA+	-
S2/cagA+/iceA1/oipA+/cagE+/babA+	5 (9.61)
S2/cagA+/iceA1/oipA+/cagE+/babA-	9 (17.30)
S2/cagA+/iceA1/oipA+/cagE-/babA+	7 (13.46)
S2/cagA+/iceA1/oipA-/cagE+/babA+	6 (11.53)
S2/cagA-/iceA1/oipA+/cagE+/babA+	6 (11.53)
S2/cagA+/iceA1/oipA+/cagE-/babA-	10 (19.23)
S2/cagA+/iceA1/oipA-/cagE-/babA+	8 (15.38)
S2/cagA+/iceA1/oipA-/cagE+/babA-	9 (17.30)
S2/cagA-/iceA1/oipA-/cagE+/babA-	10 (19.23)
S2/cagA-/iceA1/oipA-/cagE+/babA+	5 (9.61)
S2/cagA-/iceA1/oipA+/cagE-/babA+	4 (7.69)
S2/cagA-/iceA1/oipA+/cagE+/babA-	7 (13.46)
S2/cagA-/iceA1/oipA+/cagE-/babA-	10 (19.23)
S2/cagA-/iceA1/oipA-/cagE-/babA-	14 (26.92)
S2/cagA+/iceA2/oipA+/cagE+/babA+	-
S2/cagA+/iceA2/oipA+/cagE+/babA-	1 (1.92)
S2/cagA+/iceA2/oipA+/cagE-/babA+	-

(Continued)

Table 7 (Continued).

Combined Genotyping Patterns	Distribution* (%)
S2/cagA+/iceA2/oipA-/cagE+/babA+	-
S2/cagA-/iceA2/oipA+/cagE+/babA+	-
S2/cagA+/iceA2/oipA+/cagE-/babA-	1 (1.92)
S2/cagA+/iceA2/oipA-/cagE-/babA+	-
S2/cagA+/iceA2/oipA-/cagE+/babA-	-
S2/cagA-/iceA2/oipA+/cagE+/babA+	-
S2/cagA-/iceA2/oipA-/cagE+/babA+	-
S2/cagA-/iceA2/oipA+/cagE-/babA+	-
S2/cagA-/iceA2/oipA+/cagE+/babA-	-
S2/cagA-/iceA2/oipA+/cagE-/babA-	-
S2/cagA-/iceA2/oipA-/cagE-/babA-	1 (1.92)
M1a/cagA+/iceA1/oipA+/cagE+/babA+	6 (11.53)
M1a/cagA+/iceA1/oipA+/cagE+/babA-	12 (23.07)
M1a/cagA+/iceA1/oipA+/cagE-/babA+	8 (15.38)
M1a/cagA+/iceA1/oipA-/cagE+/babA+	7 (13.46)
M1a/cagA-/iceA1/oipA+/cagE+/babA+	8 (15.38)
M1a/cagA+/iceA1/oipA+/cagE-/babA-	12 (23.07)
M1a/cagA+/iceA1/oipA-/cagE-/babA+	10 (19.23)
M1a/cagA+/iceA1/oipA-/cagE+/babA-	10 (19.23)
M1a/cagA-/iceA1/oipA-/cagE+/babA-	11 (21.15)
M1a/cagA-/iceA1/oipA-/cagE+/babA+	7 (13.46)
M1a/cagA-/iceA1/oipA+/cagE-/babA+	6 (11.53)
M1a/cagA-/iceA1/oipA+/cagE+/babA-	9 (17.30)
M1a/cagA-/iceA1/oipA+/cagE-/babA-	11 (21.15)
M1a/cagA-/iceA1/oipA-/cagE-/babA-	17 (32.69)
M1a/cagA+/iceA2/oipA+/cagE+/babA+	1 (1.92)
M1a/cagA+/iceA2/oipA+/cagE+/babA-	2 (3.84)
M1a/cagA+/iceA2/oipA+/cagE-/babA+	1 (1.92)
M1a/cagA+/iceA2/oipA-/cagE+/babA+	-
M1a/cagA-/iceA2/oipA+/cagE+/babA+	2 (3.84)
M1a/cagA+/iceA2/oipA+/cagE-/babA-	2 (3.84)
M1a/cagA+/iceA2/oipA-/cagE-/babA+	-
M1a/cagA+/iceA2/oipA-/cagE+/babA-	1 (1.92)
M1a/cagA-/iceA2/oipA+/cagE+/babA+	-
M1a/cagA-/iceA2/oipA-/cagE+/babA+	-
M1a/cagA-/iceA2/oipA+/cagE-/babA+	-
M1a/cagA-/iceA2/oipA+/cagE+/babA-	1 (1.92)
M1a/cagA-/iceA2/oipA+/cagE-/babA-	2 (3.84)
M1a/cagA-/iceA2/oipA-/cagE-/babA-	3 (5.76)
M1b/cagA+/iceA1/oipA+/cagE+/babA+	2 (3.84)
M1b/cagA+/iceA1/oipA+/cagE+/babA-	5 (9.61)
M1b/cagA+/iceA1/oipA+/cagE-/babA+	2 (3.84)
M1b/cagA+/iceA1/oipA-/cagE+/babA+	2 (3.84)
M1b/cagA-/iceA1/oipA+/cagE+/babA+	2 (3.84)
M1b/cagA+/iceA1/oipA+/cagE-/babA-	6 (11.53)
M1b/cagA+/iceA1/oipA-/cagE-/babA+	3 (5.76)
M1b/cagA+/iceA1/oipA-/cagE+/babA-	4 (7.69)
M1b/cagA-/iceA1/oipA-/cagE+/babA-	5 (9.61)
M1b/cagA-/iceA1/oipA-/cagE+/babA+	1 (1.92)
M1b/cagA-/iceA1/oipA+/cagE-/babA+	-

(Continued)

Table 7 (Continued).

Combined Genotyping Patterns	Distribution* (%)
M1b/cagA-/iceA1/oipA+/cagE+/babA-	4 (7.69)
M1b/cagA-/iceA1/oipA+/cagE-/babA-	5 (9.61)
M1b/cagA-/iceA1/oipA-/cagE-/babA-	11 (21.15)
M1b/cagA+/iceA2/oipA+/cagE+/babA+	-
M1b/cagA+/iceA2/oipA+/cagE+/babA-	-
M1b/cagA+/iceA2/oipA+/cagE-/babA+	-
M1b/cagA+/iceA2/oipA-/cagE+/babA+	-
M1b/cagA-/iceA2/oipA+/cagE+/babA+	-
M1b/cagA+/iceA2/oipA+/cagE-/babA-	1 (1.92)
M1b/cagA+/iceA2/oipA-/cagE-/babA+	-
M1b/cagA+/iceA2/oipA-/cagE+/babA-	-
M1b/cagA-/iceA2/oipA+/cagE+/babA+	-
M1b/cagA-/iceA2/oipA-/cagE+/babA+	-
M1b/cagA-/iceA2/oipA+/cagE-/babA-	1 (1.92)
M1b/cagA-/iceA2/oipA-/cagE-/babA-	-
M2/cagA+/iceA1/oipA+/cagE+/babA+	4 (7.69)
M2/cagA+/iceA1/oipA+/cagE+/babA-	9 (17.30)
M2/cagA+/iceA1/oipA+/cagE-/babA+	7 (13.46)
M2/cagA+/iceA1/oipA-/cagE+/babA+	6 (11.53)
M2/cagA-/iceA1/oipA+/cagE+/babA+	5 (9.61)
M2/cagA+/iceA1/oipA+/cagE-/babA-	9 (17.30)
M2/cagA+/iceA1/oipA-/cagE-/babA+	8 (15.38)
M2/cagA+/iceA1/oipA-/cagE+/babA-	9 (17.30)
M2/cagA-/iceA1/oipA-/cagE+/babA-	9 (17.30)
M2/cagA-/iceA1/oipA-/cagE+/babA+	4 (7.69)
M2/cagA-/iceA1/oipA+/cagE-/babA+	4 (7.69)
M2/cagA-/iceA1/oipA+/cagE+/babA-	7 (13.46)
M2/cagA-/iceA1/oipA+/cagE-/babA-	9 (17.30)
M2/cagA-/iceA1/oipA-/cagE-/babA-	12 (23.07)
M2/cagA+/iceA2/oipA+/cagE+/babA+	-
M2/cagA+/iceA2/oipA+/cagE+/babA-	1 (1.92)
M2/cagA+/iceA2/oipA+/cagE-/babA+	-
M2/cagA+/iceA2/oipA-/cagE+/babA+	-
M2/cagA-/iceA2/oipA+/cagE+/babA+	-
M2/cagA+/iceA2/oipA+/cagE-/babA-	1 (1.92)
M2/cagA+/iceA2/oipA-/cagE-/babA+	-
M2/cagA+/iceA2/oipA-/cagE+/babA-	-
M2/cagA-/iceA2/oipA+/cagE-/babA+	-
M2/cagA-/iceA2/oipA+/cagE+/babA-	-
M2/cagA-/iceA2/oipA-/cagE-/babA-	1 (1.92)

Note: *Distribution was achieved based on the total numbers of 52 *H. pylori* isolates.

samples, respectively.³⁶ Moreover, Hemmatinezhad et al¹⁰ conveyed that the incidence of *H. pylori* bacteria amongst the 550 ready-to-eat food samples was 13.45% in which olive salad (36%), restaurant salad (30%), fruit salad

(28%), and soup (22%) were the most generally contaminated samples. Similarly, Ghorbani et al³¹ stated that the incidence of *H. pylori* bacteria amongst the 300 foodstuffs were 20%, in which the incidence of contamination of ready to eat fish, ham, chicken sandwich, vegetable sandwich, meat sandwich and minced meat samples were 15%, 8.33%, 5%, 45%, 20%, and 32%, respectively. Finally, Talimkhani and Mashak⁴¹ represented that the incidence of *H. pylori* bacteria in raw bovine, ovine, and caprine meat samples were 4%, 10%, and 8%, respectively. We originated that ovine meat was the most routinely contaminated samples. Similarly, Saeidi and Sheikhshahrokh (2016),⁴⁰ Talimkhani and Mashak,⁴¹ Momtaz et al,⁴² and Elhariri et al⁴³ stated the higher incidence of *H. pylori* in ovine sources. Likewise, Rahimi and Kheirabadi⁴⁴ stated that the incidence of *H. pylori* bacteria in raw bovine, ovine, caprine, buffalo, and camel milk samples were 1.41%, 12.20%, 8.70%, 23.40%, and 3.60%, respectively. Higher incidence of *H. pylori* in raw ovine meat samples may be owing to the more appropriate circumstances existing in ovine meat, such as higher fat and protein contents and water activity and also optimum pH. Additionally, ovine meat may have a higher qualification for growth and survival of *H. pylori* bacteria. Furthermore, variances in the feed of ovine with other animal species may affect the incidence rate of *H. pylori* existing in their meat. Using thorns and thistles in deserts and living away from humans and the polluted environments are the most likely reasons for the lower incidence of *H. pylori* in camel meat. Lower incidence of *H. pylori* in raw camel meat was also conveyed.^{17,37,44}

Resistance toward human and animal-based antibiotic agents was studied in the current research. *H. pylori* bacteria displayed the high incidence of resistance toward tetracycline, erythromycin, trimethoprim, levofloxacin, amoxicillin, and clarithromycin antibiotic agents. Resistance toward metronidazole, amoxicillin, levofloxacin, and clarithromycin were accompanied by the presence of *rdxA*, *pbp1A*, *gyrA*, and *cla* antibiotic resistance genes. Considerable incidence of resistance toward human-based antibiotics including erythromycin, metronidazole, levofloxacin, clarithromycin, amoxicillin, cefsulodin, furazolidone, rifampin, and spiramycin in *H. pylori* bacteria characterized their anthropogenic origin. Thus, this finding can indirectly prove that the *H. pylori* bacteria were transmitted from infected humans to meat samples through cross-contamination and meat manipulation in slaughterhouses. Extreme, unlawful, and forbidden prescription of antibiotic agents in medicine and also veterinary

caused a significant occurrence of antibiotic resistance. Diverse research on India, Iran, Taiwan, China, Nigeria, Thailand, Senegal, Saudi Arabia, Egypt, Brazil, Colombia, and Argentina showed that *H. pylori* bacteria displayed a high incidence of resistance toward tetracyclines, aminoglycosides, penicillins, metronidazole, fluoroquinolones, and macrolides,⁴⁵ which is similar to our findings. Recent surveys revealed that the incidence of resistance of *H. pylori* bacteria recovered from foodstuffs toward metronidazole, erythromycin, clarithromycin, amoxicillin, tetracycline, levofloxacin trimethoprim, furazolidone, and spiramycin antibiotic agents had ranges between 27.27–89.18%, 53.73–80.64%, 72.72–94.59%, 63.63–90.32%, 36.48–58.06%, 34.32–63.63%, 9.09–29.03%, and 9.09–16.12%, respectively.^{10,17,40,46,47} Despite the boost in importance of detection of antibiotic resistance genes, there were no previously published data on the detection of *rdxA*, *pbp1A*, *gyrA*, and *cla* antibiotic resistance genes in *H. pylori* bacteria recovered from foodstuffs. However, their detection has been done in *H. pylori* bacteria recovered from human clinical specimens.^{14,48,51}

The final part of our survey focused on the genotyping of *vacA*, *cagA*, *cagE*, *iceA*, *oipA*, and *babA* alleles of the *H. pylori* bacteria. We also originated that *vacA* *s1a*, *s2*, *m1a*, and *m2*, and *iceA1* and *cagA*, *s1am1a*, *s2m1a*, *s1am2*, *s2m2*, and *s1a/cagA-/iceA1/oipA-/cagE-/babA-*, *m1a/cagA-/iceA1/oipA-/cagE-/babA-*, *s2/cagA-/iceA1/oipA-/cagE-/babA-*, *s1a/cagA+/iceA1/oipA+/cagE-/babA-*, *m1a/cagA+/iceA1/oipA+/cagE+/babA-*, *m1a/cagA+/iceA1/oipA+/cagE-/babA-*, *m2/cagA-/iceA1/oipA-/cagE-/babA-*, *s1a/cagA+/iceA1/oipA+/cagE+/babA-*, *s1a/cagA-/iceA1/oipA-/cagE+/babA-*, *s1a/cagA-/iceA1/oipA+/cagE-/babA-*, *s1b/cagA-/iceA1/oipA-/cagE-/babA-*, *m1a/cagA-/iceA1/oipA-/cagE+/babA-*, *m1a/cagA-/iceA1/oipA+/cagE-/babA-*, *m1b/cagA-/iceA1/oipA-/cagE-/babA-*, *s1a/cagA+/iceA1/oipA-/cagE+/babA-*, *s2/cagA+/iceA1/oipA+/cagE-/babA-*, *s2/cagA-/iceA1/oipA-/cagE+/babA-*, *s2/cagA-/iceA1/oipA+/cagE-/babA-*, *m1a/cagA+/iceA1/oipA-/cagE-/babA+*, and *m1a/cagA+/iceA1/oipA-/cagE+/babA-* were the most generally perceived genotypes amongst the *H. pylori* bacteria. The boost incidence of *vacA*, *cagA*, *iceA1*, *oipA*, *cagE* and *babA2* genotypes was also conveyed in the *H. pylori* bacteria recovered from clinical samples of human and animal species.^{42,52,54} Khaji et al⁵⁵ conveyed that *vacA* *s1a* (91.66%), *m1a* (61.61%), *s2* (36.66%), and *m2* (31.66%) were the most generally perceived genotypes amongst the *H. pylori* bacteria recovered from raw milk of animal species. They also showed that *s1am1a* (41.66%), *s2m1a* (25%),

s1am2 (16.66%), and *s2m2* (13.33%) were the most generally perceived genotyping patterns amongst the *H. pylori* isolates. Ranjbar et al (2018)⁴⁷ conveyed that *vacA s1a* (83.58%), *m1a* (80.59%), *s2* (77.61%) and *m2* (68.65%), *cagA* (73.13%) and *babA2* (44.77%) were the most generally perceived genotypes amongst the *H. pylori* bacteria recovered from diverse kinds of raw milk samples. They showed that the distribution of *s1am1a*, *s2m1a*, *s1am2* and *s2m2* genotyping patterns and *cagA*-, *oipA*-, and *babA2*- genotypes were 56.71%, 56.71%, 43.28%, and 43.28% and 26.86%, 62.68%, and 55.22%, respectively. Additionally, amongst all of the detected combined genotypes, *s1a/cagA+/iceA1/oipA-/babA2-* (28.35%), *m1a/cagA+/iceA1/oipA-/babA2-* (28.35%), *s2/cagA+/iceA1/oipA-/babA2-* (26.86%), *s1a/cagA+/iceA1/oipA-/babA2+* (25.37%), *m1a/cagA+/iceA1/oipA-/babA2+* (25.37%), *s2/cagA+/iceA1/oipA-/babA2+* (23.88%), *s1a/cagA+/iceA1/oipA+/babA2-* (22.38%), and *m2/cagA+/iceA1/oipA-/babA2+* (22.38%) had the uppermost distribution. Hemmatinezhad et al⁵⁶ stated that *vacA s1a* (78.37%), *vacA m2* (75.67%), *vacA m1a* (51.35%), and *cagA* (41.89%) alleles, *s1am2* (70.27%), *s1am1a* (39.18%), and *m1am2* (31.08%) genotypes, and *s1a/cagA+/iceA1/oipA-* (12.16%), *s1a/cagA+/iceA1/oipA+* (10.81%), *s1a/cagA-/iceA1/oipA+* (10.81%), *s1b/cagA+/iceA1/oipA-* (9.45%), *m2/cagA+/iceA1/oipA+* (9.45%), *m2/cagA+/iceA1/oipA-* (9.45%), *m2/cagA-/iceA1/oipA+* (9.45%), and *m2/cagA-/iceA1/oipA-* (9.45%) combined genotypic patterns were the most generally perceived in the *H. pylori* bacteria recovered from ready to eat food. According to Talimkhani and Mashak,⁴¹ *vacA s1a* (87.50%), *vacA m1a* (87.50%), *vacA s2* (82.50%), *cagA* (80%), and *vacA m2* (62.50%) alleles and *s1am1a* (62.50%), *s2m1a* (55%), *s1am2* (50%), *s2m2* (45%), and *m1am2* (42.50%) genotypes were the most generally perceived in *H. pylori* bacteria recovered from meat, milk, and vegetable samples. In studies conducted by Gilani et al^{40,56} *s1am1a*, *s1am1b*, and *s2m1a* were the most generally perceived genotypes amongst the *H. pylori* bacteria recovered from raw meat and meat products. There were no previous data on detection of *cagE* genotypes amongst the *H. pylori* bacteria recovered from food samples. The presence of *vacA*, *iceA*, *oipA*, *cagA*, *cagE* and *babA2* genotypes in the *H. pylori* isolates may cause certain facilities for bacterial adhesion to gastric epithelial cells, interleukin-8 and -10 and cytotoxin secretion and occurrence of inflammation, vacuolization, apoptosis of gastric epithelial cells, and even peptic ulceration in individuals who consume studied contaminated meat samples.

Absolutely, impact of food-borne microbes, particularly bacteria, in occurrence of food-borne diseases has been measured in Iran and diverse surveys have been conducted in this field.⁵⁷⁻⁷⁴

Conclusions

In conclusion, we documented extensive delivery of virulent and resistant *H. pylori* bacteria in raw camel, caprine, ovine, bovine, and buffalo meat samples. Boost incidence of *H. pylori* bacteria in raw meat magnifies that raw meat, particularly raw ovine meat, may be the natural reservoirs of *H. pylori*. We also originated that *vacA*, *cagA*, *iceA*, and *babA2* alleles were predominant amongst the *H. pylori* isolates. In keeping with this, *cagE*-, *babA2*-, and *oipA*- *H. pylori* bacteria had the higher distribution. Similarities in the genotyping pattern of *H. pylori* bacteria between numerous meat sources signify their same route of contamination. *H. pylori* isolates displayed a high incidence of resistance toward tetracycline, erythromycin, trimethoprim, levofloxacin, amoxicillin, and clarithromycin (61.53%) antibiotic agents. The phenotypic pattern of antibiotic resistance was also confirmed by the genotypic pattern, with considerable distribution of *rdxA*, *pbp1A*, *gyrA*, and *cla* antibiotic resistance genes. Furthermore, the high incidence of multi-drug resistant *H. pylori* bacteria displays that raw meat of animal species may be a reservoir of antibiotic resistant *H. pylori*. Further research should be performed to determine the probable relationships between the presence of genotypes, antibiotic resistance, and antibiotic resistance genes. Additionally, conduction of comprehensive research is essential to determine molecular genetic homology of *H. pylori* bacteria recovered from raw meat of animal species and those of human clinical specimens.

Ethics Criteria

The study was approved by the Ethical Council of Research of the Faculty of Veterinary Medicine, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran.

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Disclosure

The authors report no conflicts of interest.

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