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

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Background: Foodstuffs with animal origins, particularly meat, are likely reservoirs of \textit{Helicobacter pylori}. Purpose: An existing survey was accompanied to assess phenotypic and genotypic profiles of antibiotic resistance and genotyping of \textit{vacA}, \textit{cagA}, \textit{cagE}, \textit{iceA}, \textit{oipA}, and \textit{babA2} alleles amongst the \textit{H. pylori} bacteria recovered from raw meat.

Methods: Six-hundred raw meat samples were collected and cultured. \textit{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 \textit{H. pylori}. Raw ovine meat (13.07%) had the uppermost contamination. \textit{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 \textit{H. pylori} bacteria had at least resistance toward one antibiotic, although incidence of resistance toward more than eight antibiotics was 28.84%. Total distribution of \textit{rdxA}, \textit{php1A}, \textit{gyrA}, and \textit{cla} antibiotic resistance genes were 59.61%, 51.92%, 69.23%, and 65.38%, respectively. \textit{VacA} \textit{s1a} (84.61%), \textit{s2} (76.92%), \textit{m1a} (50%), \textit{m2} (39.13%), \textit{iceA1} (38.46%), and \textit{cagA} (55.76%) were the most generally perceived alleles. \textit{Slam1a} (63.46%), \textit{s2m1a} (53.84%), \textit{slam2} (51.92%), and \textit{s2m2} (42.30%) were the most generally perceived genotyping patterns. Frequency of \textit{cagA}-, \textit{oipA}-, and \textit{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 \textit{H. pylori} bacteria was determined. \textit{VacA} and \textit{cagA} genotypes had the higher incidence. \textit{CagE}-, \textit{babA2}-, and \textit{oipA}- \textit{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: \textit{Helicobacter pylori}, antibiotic resistance, genotyping, raw meat


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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.\textsuperscript{1} Reversely, raw meat is not unavoidably safe, as evidenced by considerable rates of foodborne diseases accompanying with its consumption.\textsuperscript{2} Similarly, several outbreaks of foodborne diseases have been conveyed owing to the consumption of contaminated meat samples.\textsuperscript{2}
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. The main reservoir of H. pylori bacteria is the human stomach. In keeping with this, foods with animal origins, particularly meat, may play an imperative portion in transmission of H. pylori infections to humans. 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. Additionally, the role of meat consumption as a risk factor for occurrence of H. pylori infections has been conveyed. Likewise, the bacterium has been recovered from diverse kinds of foods with animal origins.

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). 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. CagE gene was found to serve as an improved biomarker of an intact cag-PAI in patients with severe gastrointestinal disorders. BabA2 gene mediates adherence of H. pylori to human Lewis b blood-group antigens on gastric epithelial cells. 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. The iceA gene was detected in the H. pylori recovered from patients with gastrointestinal disorders. There are at least two alleles of iceA: iceA1, and iceA2. The relationship between H. pylori iceA1 and iceA2 and clinical outcomes has been addressed by some researchers. The presence of these alleles has been conveyed in different research conducted on diverse kinds of foods with animal origins. 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. 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. The presence of certain antibiotic resistance genes, particularly rdxA, pflA, 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. 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. 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 |
|-------|--------------------------|----------------------|-------------------------------|--------------|
| vacA s,a | F: CTCTCGCTTTAGTAGGAGC R: CTGCTTGAATGCGCCAAAC | 213 | 5 µL PCR buffer 10X 1.5 mM MgCl₂ 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 |
| vacA s,b | F: AGCGCCATAACCCGCAAGGAG CTGCTTGAATGCGCCAAAC | 187 | | |
| vacA s,c | F: CTCTCGCTTTAGTGGGGYT R: CTGCTTGAATGCGCCAAAC | 213 | | |
| vacA s2 | F: GCTAACACGCCAAATGATCC R: CTGCTTGAATGCGCCAAAC | 199 | | |
| vacA m1,a | F: GGTCAAAATGCGGTCATGG R: CCATTGGTACCTGTAGAAAC | 290 | | |
| vacA m1,b | F: GGCCCCAATGCAGTCATGGA R: GCTGTTAGTGCCTAAAGAAGCAT | 291 | | |
| vacA m2 | F: GGAAGCCCCAGGAAACATTTG R: CATAACTAGCGCCTTGCA | 352 | | |
| cagA | F: GATAACAGCCAAGCTTTTGAGG R: CTGCAAAAGATTGTTTGGCAA | 300 | 5 µL PCR buffer 10X 2 mM MgCl₂ 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: 72°C; 10 min |
| iceA | IceA1 F: GGTGTTTTAACCAACTATATCGCAG R: CTATAGCCACTYTCTTTGCA | 247 | 5 µL PCR buffer 10X 2 mM MgCl₂ 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: 72°C; 10 min |
| iceA2 | F: GTTGGGTATATCACAATTTAT R: TTRCCCTATTTTCTAGTAGGT | 229/334 | | |
| OipA | F: GTTGGATGTGCAATGGGATT R: GTGCATCTCTTTGCGCTTTT | 401 | 5 µL PCR buffer 10X 2 mM MgCl₂ 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: 72°C; 10 min |
| CagE | F: TTGAAAACTTCAAGGATAGGATAGGAC R: GCCTAGCCTTAGTACGACC | 500 | 5 µL PCR buffer 10X 2 mM MgCl₂ 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: 72°C; 5 min |

(Continued)
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 1** (Continued).  

| Genes | Primer Sequence (5ʹ-3ʹ) | Size of Product (Bp) | Volume of PCR Reaction (50 µl) | PCR Programs |
|-------|-------------------------|---------------------|-------------------------------|--------------|
| BabA2 | F: CCAAACGAAACAAAAAGCGT  
       | R: GCTTGTGAAAAAGCGCTGT | 105–124 | 5 µL PCR buffer 10X  
       | 2 mM MgCl₂  
       | 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 |
| rdxA  | Metronidazole  
       | F: AATTGGATCGTGCGGCA  
       | R: GAAAGCTGTGAAAAAACCCT | 581 | 5 µL PCR buffer 10X  
       | 2 mM MgCl₂  
       | 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; 60 s  
       | 55°C; 30 s  
       | 72°C; 60 s  
       | 1 cycle:  
       | 72°C; 10 min |
| pbp1A | Amoxicillin  
       | F: GCGACAATAAGAGTGGCA  
       | R: TGCGAACACCCTTTAAA T | 2,300 | 5 µL PCR buffer 10X  
       | 2 mM MgCl₂  
       | 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:  
       | 94°C; 60 s  
       | 54°C; 60 s  
       | 72°C; 5 min  
       | 1 cycle:  
       | 72°C; 10 min |
| gyrA  | Fluoroquinolone  
       | F: TTTTCTATTCCATAGGCGGT  
       | R: GCAGACGCTTGTGATAAATA | 2,300 | 5 µL PCR buffer 10X  
       | 2 mM MgCl₂  
       | 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  
       | 47°C; 30 s  
       | 72°C; 30 s  
       | 1 cycle:  
       | 72°C; 5 min |
| cla   | Clarithromycin  
       | F: AGTGGGACCTAAGGCGGAG  
       | R: AGGTCCTGCAAGGGTGCTTG | 700 | 5 µL PCR buffer 10X  
       | 2 mM MgCl₂  
       | 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 |
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 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%), m1a/cagA+/iceA1/oipA+/cagE+/babA- (21.15%), m1a/cagA+/iceA1/oipA+/cagE+/babA+ (21.15%), m1b/cagA+/iceA2/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%), 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-, s1a/cagA+/iceA2/oipA+/cagE+/babA-, s1a/cagA+/iceA2/oipA+/cagE-/babA+, s1b/cagA+/iceA1/oipA+/cagE+/babA-, s1b/cagA+/iceA1/oipA+/cagE-/babA-, s1b/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-, s1a/cagA+/iceA2/oipA+/cagE/babA-, s1a/cagA+/iceA2/oipA+/cagE+/babA+, s1b/cagA+/iceA1/oipA+/cagE+/babA-, s1b/cagA+/iceA1/oipA+/cagE-/babA-, s1b/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-, s1a/cagA+/iceA2/oipA+/cagE/babA-, s1a/cagA+/iceA2/oipA+/cagE+/babA+, s1b/cagA+/iceA1/oipA+/cagE+/babA-, s1b/cagA+/iceA1/oipA+/cagE-/babA-, s1b/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.

**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 *H. pylori* Bacteria) | Metronidazole | Amoxicillin | Fluoroquinolone | Clarithromycin |
|---------------------------------------------------|---------------|-------------|----------------|----------------|
|                                                   | rdxA          | pbp1A       | gyrA           | cla            |
| Bovine (8)                                        | 4 (50)        | 5 (62.50)   | 4 (50)         | 5 (62.50)      |
| Owine (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)     |
+/cagE+/babA+, s1b/cagA-/iceA1/oipA-/cagE+/babA+, s1c/cagA+/iceA1/oipA-/cagE+/babA+, s1c/cagA+/iceA1/oipA- 
+/cagE+/babA-, s1b/cagA-/iceA1/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+, m1a/cagA+/iceA2/oipA+/cagE+/babA+, m1a/cagA+/iceA2/oipA+/cagE+/babA+, m1a/cagA+/iceA2/oipA+/cagE+/babA+, m2/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. Furthermore, bacterial transmission between persons ensues through the oral–oral and oral–fecal routes. However, oral–fecal transmission has additional implications, since *H. pylori* may occur in food and water supplies subsequent to fecal contamination. Additionally, isolation of *H. pylori* from raw vegetables, meat, salads, ready to eat foods, and milk 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. Saeidi and Sheikhshahrokh 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 al 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 *H. pylori* Bacteria) | Genotyping pattern (%) | iceA1 | iceA2 | OipA | CagE | BabA2 |
|---------------------------------------------------|------------------------|-------|-------|------|------|-------|
| Bovine (8)                                        |                        | 5 (62.50) | 3 (37.50) | 2 (25) | 6 (75) | 3 (37.50) |
| Ovine (17)                                        |                        | 2 (11.76) | 1 (5.88) | 12 (70.58) | 1 (11.76) | 70.58 |
| Caprine (15)                                      |                        | 4 (23.52) | 4 (23.52) | 14 (82.35) | 10 (58.82) | 70.58 |
| Buffalo (9)                                       |                        | 4 (44.44) | 11 (73.33) | 13 (76.47) | 4 (23.52) | 82.35 |
| Camel (3)                                         |                        | 2 (33.33) | 1 (33.33) | 3 (100) | 2 (55.55) | 66.66 |
| Total (52)                                        |                        | 23 (44.23) | 5 (9.61) | 14 (26.92) | 3 (5.76) | 55.76 |

*Note: N of *H. pylori* Bacteria refers to the number of bacteria isolated from each type of raw meat sample.*
| Combined Genotyping Patterns | Distribution* (%) |
|-----------------------------|-------------------|
| S1a/cagA+/iceA2/oipA+/cagE+-babA- | 1 (1.92) |
| S1a/cagA+/iceA2/oipA+/cagE-/babA+ | 2 (3.84) |
| S1a/cagA+/iceA2/oipA+/cagE+/babA- | 1 (1.92) |
| S1a/cagA+/iceA2/oipA+/cagE+/babA+ | 1 (1.92) |
| S1b/cagA+/iceA2/oipA+/cagE-/babA- | 6 (11.53) |
| S1b/cagA+/iceA2/oipA+/cagE+/babA+ | 3 (5.76) |
| S1b/cagA+/iceA2/oipA+/cagE-/babA+ | 5 (9.61) |
| S1b/cagA+/iceA2/oipA+/cagE+/babA+ | 1 (1.92) |
| S1b/cagA+/iceA2/oipA+/cagE+/babA- | 2 (3.84) |
| S1b/cagA+/iceA2/oipA+/cagE+/babA+ | 5 (9.61) |
| S1b/cagA+/iceA2/oipA+/cagE-/babA+ | 4 (7.69) |
| S2/cagA+/iceA2/oipA+/cagE+/babA+ | 6 (11.53) |
| S2/cagA+/iceA2/oipA+/cagE+/babA+ | 6 (11.53) |

<Continued>

| Combined Genotyping Patterns | Distribution* (%) |
|-----------------------------|-------------------|
| S1b/cagA+/iceA2/oipA+/cagE+/babA- | 2 (3.84) |
| S1b/cagA+/iceA2/oipA+/cagE+/babA+ | 1 (1.92) |
| S1b/cagA+/iceA2/oipA+/cagE+/babA- | 2 (3.84) |
| S1b/cagA+/iceA2/oipA+/cagE+/babA+ | 2 (3.84) |
| S1b/cagA+/iceA2/oipA+/cagE+/babA- | 3 (5.76) |
| S1b/cagA+/iceA2/oipA+/cagE+/babA+ | 5 (9.61) |
| S2/cagA+/iceA2/oipA+/cagE+/babA+ | 5 (9.61) |
| S2/cagA+/iceA2/oipA+/cagE+/babA+ | 9 (17.30) |
| S2/cagA+/iceA2/oipA+/cagE+/babA+ | 7 (13.46) |
| S2/cagA+/iceA2/oipA+/cagE+/babA+ | 6 (11.53) |
| S2/cagA+/iceA2/oipA+/cagE+/babA+ | 6 (11.53) |
| S2/cagA+/iceA2/oipA+/cagE+/babA+ | 10 (19.23) |
| S2/cagA+/iceA2/oipA+/cagE+/babA+ | 8 (15.38) |
| S2/cagA+/iceA2/oipA+/cagE+/babA+ | 9 (17.30) |

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

Table 7 (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+ | 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+/iceA1/oipA+/cagE+/babA+ | 1 (1.92)          |
| M1a/cagA+/iceA1/oipA+/cagE+/babA+ | 2 (3.84)          |
| M1a/cagA+/iceA1/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+ | –                 |
| 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+ | 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+ | –                 |

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

samples, respectively. Moreover, Hemmatinezhad et al 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
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 Mashak\(^\text{41}\) 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 Sheikhshahrok\hbox{h}\(\text{16}\),\(^\text{40}\) Talimkhani and Mashak,\(^\text{41}\) Momtaz et al,\(^\text{42}\) and Elhariri et al\(^\text{43}\) stated the higher incidence of *H. pylori* in ovine sources. Likewise, Rahimi and Kheirabadi\(^\text{44}\) 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.\(^\text{17,37,44}\)

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,\(^\text{45}\) 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.\(^\text{10,17,40,46,47}\) 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.\(^\text{14,48,51}\)

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, slam1a, s2m1a, slam2, s2m2*, and *s1a/cagA-/iceA1/oipA-/cagE-/babA-, m1a/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-, ml1a/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-, s1a/cagA+/iceA1/oipA+/cagE+/babA-, s1b/cagA+/iceA1/oipA+/cagE+/babA-, m1a/cagA+/iceA1/oipA+/cagE+/babA-, s1a/cagA+/iceA1/oipA+/cagE+/babA-, s2/cagA-/iceA1/oipA+/cagE-/babA-, ml1a/cagA+/iceA1/oipA+/cagE+/babA-, m1b/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.\(^\text{42,52,54}\) Khaji et al\(^\text{45}\) 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 *slam1a* (41.66%), *s2m1a* (25%),...
slam2 (16.66%), and s2m2 (13.33%) were the most generally perceived genotyping patterns amongst the H. pylori isolates. Ranjar et al (2018) 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 slam1a, s2m1a, slam2 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 al stated that vacA s1a (78.37%), vacA m2 (75.67%), vacA m1a (51.35%), and cagA (41.89%) alleles, slam2 (70.27%), slam1a (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%), 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, vacA s1a (87.50%), vacA m1a (87.50%), vacA s2 (82.50%), cagA (80%), and vacA m2 (62.50%) alleles and slam1a (62.50%), s2m1a (55%), slam2 (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 slam1a, slam1b, 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, levofoxacin, 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, ppa1A, 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.
References

1. Wyness L. The role of red meat in the diet: nutrition and health benefits. *Proc Nutr Soc.* 2016;75(3):227–232. doi:10.1017/S0029665115004267
2. Omer MK, Alvarez-Ordonez A, Prieto M, Skjerve E, Asehnun T, Alveiski OA. A systematic review of bacterial foodborne outbreaks related to red meat and meat products. *Foodborne Pathog Dis.* 2018;15(10):598–611. doi:10.1097/FPD.2017.2393
3. Crowe SE. Helicobacter pylori infection. *New England J Med.* 2019;380(12):1158–1165. doi:10.1056/NEJMcp1710945
4. Quaglia N, Dambrosio A. Helicobacter pylori: a foodborne pathogen? *World J Gastroenterol.* 2018;24(31):3472–3487. doi:10.3748/wjg.v24.i31.3472
5. Zamani M, Vahedi A, Maghdour Z, Shokri-Shirvani J. Role of food in environmental transmission of Helicobacter pylori. *Caspian J Int Med.* 2017;8(3):146.
6. Van Hecke T, Van Camp J, De Smet S. Oxidation during digestion of meat: interactions with the diet and helicobacter pylori gastritis, and implications on human health. *Comp Rev Food Sci Food Safe.* 2017;16(2):234–233. doi:10.1111/1541-4337.12248
7. Xia Y, Meng G, Zhang Q, et al. Dietary patterns are associated with Helicobacter pylori infection in Chinese adults: a cross-sectional study. *Sci Report.* 2016;6:32334. doi:10.1038/srep32334
8. Sedaghat H, Moniri R, Jamali R, et al. Prevalence of Helicobacter pylori vacA, cagA, cagE, iceA, babA2, and oipA genotypes in patients with upper gastrointestinal disorders. *Iran J Microbiol.* 2014;6(1):14.
9. Akeel M, Shehata A, Elhafey A, et al. Helicobacter pylori vacA, cagA and iceA genotypes in dyspeptic patients from southwestern region, Saudi Arabia: distribution and association with clinical findings and histopathological changes. *BMJ Gastroenterol.* 2019;19(1):16. doi:10.1186/s12876-019-0934-z
10. Hemmatinezhad B, Montaz H, Rahimi E. VacA, cagA, iceA and oipA genotypes status and antimicrobial resistance properties of Helicobacter pylori isolated from various types of ready to eat foods. *Ann Clin Microbiol Antimicrob.* 2014;13:32.
11. Suzuki S, Esaki M, Kusano C, Ikehara H, Gotoda T. Development of Helicobacter pylori from sheep: genotyping and antimicrobial resistance properties of Helicobacter pylori isolated from sheep in the gastric mucosa. *Veterinary Microbiology.* 2019;233. doi:10.1016/j.vetmic.2019.06.016
12. Suvoldi A, Carraza E, Graham DY, Conti M, Tacconelli E. Prevalence of antibiotic resistance in Helicobacter pylori: a systematic review and meta-analysis in World Health Organization regions. *Gastroenterol.* 2018;155(5):1372–1382. doi:10.1053.j.gastro.2018.07.007
13. Yousefi-Avarvand A, Vaez H, Tafaghodi M, Rassabghali G, De Paoli P. Heterogeneity of cag genotypes in Helicobacter pylori isolates from human biopsy specimens. *J Clin Microbiol.* 2003;41(3):976–980. doi:10.1128/JCM.41.3.976-980.2003
14. Debets-Ossenkopp Y, Sparrius M, Kusters J, Vandenbroucke-Grauls C. Mechanism of clarithromycin resistance in clinical isolates of Helicobacter pylori. *FEMS Microb Lett.* 1996;142(1):37–42. doi:10.1111/j.1574-6968.1996.tb08404.x
15. Sanger F, Coulson AR. A rapid method for determining sequences in DNA by primed synthesis with DNA polymerase. *J Molecular Biol.* 1975;94(3):441–448. doi:10.1016/0022-2836(75)90213-2
16. Tankovik J, Lascols C, Scuro Q, Petit J-C, Sousy C-J. Single and double mutations in gyrA but not in gyrB are associated with low-and high-level fluoroquinolone resistance in Helicobacter pylori. *Antimicrob Agents Chemother.* 2003;47(12):3942–3944. doi:10.1128/AAC.47.12.3942-3944.2003
17. Versalovic J, Shortridge D, Kibler K, et al. Mutations in 23S rRNA are associated with clarithromycin resistance in Helicobacter pylori. *Antimicrob Agents Chemother.* 1996;40(2):477–480. doi:10.1128/AAC.40.2.477-480.1996
18. Ho S-A, Hoyle J, Lewis F, et al. Direct polymerase chain reaction test for detection of Helicobacter pylori in humans and animals. *J Clin Microbiol.* 1991;29(11):2543–2549.
19. Andrews J. BSAC Disc Diffusion Method Antimicrob. 2.1.4 ed. Birmingham, UK: British Society for Antimicrobial Chemotherapy; 2003.
20. CSLI. Clinical and Laboratory Standard Institute. In: *Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria.* Wayne, PA: National Committee for Clinical Laboratory Standards; 2015:M45.
21. Yamazaki S, Yamakawa A, Okuda T, et al. Distinct diversity of vacA, cagA, and cagE genotypes of Helicobacter pylori associated with peptic ulcer in Japan. *J Clin Microbiol.* 2005;43(8):3906–3916. doi:10.1128/JCM.43.8.3906-3916.2005
22. Wang J, Chi DS, Laffan JJ, et al. Comparison of cytotoxin gene types of Helicobacter pylori in stomach and saliva. *Digest Dis Sci.* 2002;47(8):1850–1856. doi:10.1023/A:1016417200611
23. Poms RE, Tatini SR. Survival of Helicobacter pylori in ready-to-eat foods at 4 C. *Int J Food Microbiol.* 2001;63(3):281–286. doi:10.1016/S0168-1605(00)00441-4
36. Meng X, Zhang H, Law J, Tsang R, Tsang T. Detection of Helicobacter pylori from food sources by a novel multiplex PCR assay. J Food Safe. 2008;28(4):609–619. doi:10.1111/j.1745-4658.2008.00135.x

37. Esmaeiligoudarzi D, Tameshkel FS, Ajdarkosh H, Arsalani M, Sohani MH, Behnood V. Prevalence of Helicobacter pyloriiiranian milk and dairy products using culture and ureC based-PCR techniques. Biomed Pharmacol J. 2015;8(1):179–183. doi:10.13005/bp/597

38. Talaei R, Souod N, Montzaz H, Dabiri H. Milk of livestock as a possible transmission route of Helicobacter pylori infection. Gastroentrol Hepatol Bed Bench. 2015;8(Suppl1):S30.

39. Saedi E, Sheikhhsabzrokh A. VacA genotype status of Helicobacter pylori isolated from foods with animal origin. Biomed Res Int. 2016;2016.

40. Gilani A, RazaviV, Rokni N, Rahimi E. VacA and cagA genotypes and antimicrobial resistance properties of Helicobacter pylori strains isolated from meat products in Isfahan province, Iran. Iran J Vet Res. 2017;18(2):97.

41. Talinkhani A, Mashak Z. Prevalence and genotyping of Helicobacter pylori isolated from meat, milk and vegetable in Iran. Jundishapur J Microbiol. 2017;10:11. doi:10.5812/jjm

42. Montzaz H, Dabiri H, Souod N, Gholami M. Study of Helicobacter pylori genotype status in cows, sheep, goats and humans beings. BMC Gastroenterol. 2014;14(1):61. doi:10.1186/1471-230X-14-61

43. Elhariri M, Hamza D, Elhelw R, Hamza E. Occurrence of cagA+ vacA s1a m1 in Helicobacter pylori in farm animals in Egypt and ability to survive in experimentally contaminated UHT milk. Sci Reports. 2018;8(1):14260. doi:10.1038/s41598-018-32671-0

44. 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. doi:10.1089/fpd.2011.1060

45. Hunt R, Xiao S, Megraud F, et al. Helicobacter pylori in developing countries. J Gastrointestin Liver Dis. 2011;20(3):299–304.

46. Ranjbar R, Yaroddahi Farsani F, Safarpoor Dehkordi F. Antimicrobial resistance and genotyping of vacA, cagA, and iceA alleles of the Helicobacter pylori strains isolated from traditional dairy products. J Food Saf. 2019;39(2):12594. doi:10.1111/jfs.12594

47. Ranjbar R, Farsani FY, Dekhordi FS. Phenotypic analysis of antibiotic resistance and genotype study of the vacA, cagA, iceA, oipA and babA genotypes of the Helicobacter pylori strains isolated from raw milk. Antimicrob Res Infect Control. 2018;7(1):115. doi:10.1186/s13756-018-0409-y

48. Launier F, Imkamp F, Lehours P, et al. Genetic determinants and prediction of antibiotic resistance phenotypes in Helicobacter pylori. J Clin Med. 2019;8(1):53. doi:10.3390/jcm8010053

49. Amin M, Shajesteh AA, Serajian A, Goodarzi H. Assessment of metronidazole and clarithromycin resistance among Helicobacter pylori isolates of Ahvaz (Southwest of Iran) during 2015-2016 by conventional vs real-time PCR for detection of bovine herpes virus type 1 in aborted bovine, buffalo and camel foetuses. Colombian J Vet Med.2015;16(2):102–111.

50. Rahimi E, Yazdanpour S, Dehkordi FS, Shayan S, Montzaz H. Prevalence of Yersinia species in traditional and commercial dairy products in Isfahan Province, Iran. Jundishapur J Microbiol. 2014;7(4):e9249.

51. Dehkordi FS, Khamesipour F, Momeni M. Brucella abortus and Brucella melitensis in Iranian bovine and buffalo semen samples: The first clinical trial on seasonal, Senile and geographical distribution using culture, conventional and real-time polymerase chain reaction assays. Kafras Univ Vet Fak Derg. 2014;20(6):821–828.

52. Dehkordi FS, Haghighi N, Montzaz H, Rafsanjani MS, Momeni M. Conventional vs real-time PCR for detection of bovine herpes virus type 1 in aborted bovine, buffalo and camel foetuses. Bulgarian J Vet Med.2013;16(2):102–111.

53. Rahimi E, Yazdanpour S, Dehkordi FS. Detection of Toxoplasma gondii antibodies in various poultry meat samples using enzyme linked immunoblot sorbent assay and its confirmation by polymerase chain reaction. J Pure Appl Microbiol.2014;8(1):421–427.

54. Dehkordi FS, Valizadeh Y, Birgani T, Dehkordi K. Prevalence study of Brucella melitensis and Brucella abortus in cow's milk using dot enzyme linked immunoblot sorbent assay and duplex polymerase chain reaction. J Pure Appl Microbiol. 2014;8:1065–1069.

55. Ranjbar R, Seif A, Safarpoor Dekhordi F. Prevalence of Antibiotic Resistance and Distribution of Virulence Factors in the Shiga Toxigenic Escherichia coli Recovered from Hospital Food. Jundishapur J Microbiol. 2019;12(4):e82659.

56. Nejat S, Montzaz H, Yadegari M, Nejat S, Safarpoor Dekhordi F, Khamesipour F. Seasonal, Geographical, Age and Breed Distributions of Equine Viral Arteritis in Iran. Kafras Univ Vet Fak Derg. 2015; 21(1):111–116.

57. Ranjbar R, Safarpoor Dekhordi F, Sakhai Shahrzea MH, Rahimi E. Prevalence, identification of virulence factors, O-serogroups and antibiotic resistance properties of Shiga-toxin producing Escherichia coli strains isolated from raw milk and traditional dairy products. Antimicrob Res Infect Control. 2018;7:35.
70. Momtaz H, Safarpoor Dehkordi F, Taktaz T, Rezvani A, Yarali S. Shiga Toxin-Producing Escherichia coli Isolated from Bovine Mastitic Milk: Serogroups, Virulence Factors, and Antibiotic Resistance Properties. *Sci World J*. 2012;2012:618709.

71. Dehkordi AH, Khaji L, Shahreza MS, et al. One-year prevalence of antimicrobial susceptibility pattern of methicillin-resistant Staphylococcus aureus recovered from raw meat. *Trop Biomed.* 2017;34(2):396–404.

72. Safarpoor Dehkordi F, Gandomi H, Akhondzadeh Basti A, Misaghi A, Rahimi E. Phenotypic and genotypic characterization of antibiotic resistance of methicillin-resistant Staphylococcus aureus isolated from hospital food. *Antimicrob Res Infect Control*. 2017;6:104.

73. Momtaz H, Dehkordi FS, Rahim E., Asgarifar A, Momeni M. Virulence genes and antimicrobial resistance profiles of Staphylococcus aureus isolated from chicken meat in Isfahan province, Iran. *J Appl Poult Res*. 2013;22(4):913–921.

74. Ranjbar R, Masoudimanesh M, Dehkordi FS, Jonaidi-Jafari N, Rahimi E. Shiga (Vero)-toxin producing Escherichia coli isolated from the hospital foods; virulence factors, o-serogroups and antimicrobial resistance properties. *Antimicrob Res Infect Control*. 2017;6:4.