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Original article

# *Helicobacter pylori* in a poultry slaughterhouse: Prevalence, genotyping and antibiotic resistance pattern

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A B S T R A C T

Although *Helicobacter pylori* (*H. pylori*) is a highly significant pathogen, its source remains unclear. Many people consume chicken daily as a source of animal protein worldwide; thus, hygienic methods of supplying chickens for consumption are critical for public health. Therefore, our study examined the distribution of the *glmM* (*ureC*), *babA2*, *vacA* and *cagA* virulence genes in *H. pylori* strains in chicken meat and giblets (gizzards and livers) and the resistance of the strains to various antibiotics. Ninety chicken meat, gizzard and liver samples were obtained from a semi-automatic abattoir in Sadat City, Egypt, and were cultured and preliminarily analyzed using biochemical tests. The presence of the *ureC*, *babA2*, *vacA* and *cagA* genotypes was tested for in samples positive for *H. pylori* by multiplex polymerase chain reaction (Multiplex-PCR). The resistance of *H. pylori* to various antimicrobial drugs was tested using the disc diffusion method. In total, 7 of the 90 chicken samples were positive for *H. pylori* (7.78%); in 3/7 (42.85%) samples, the bacteria were found in the chicken liver, while the bacteria were found in the meat in 2/7 (28.57%) and in the gizzard in 2/7 (28.57%) samples. The total prevalence of both the *ureC* and *babA2* genes in the isolated *H. pylori* strains was 100%, while the prevalence of the *vacA* and *cagA* genes was 57.1% and 42.9%, respectively. The resistance of *H. pylori* to the antibiotics utilized in our study was 100% for streptomycin; 85.7% for amoxicillin and penicillin; 71.4% for oxytetracycline, nalidixic acid and ampicillin; 57.1% for sulfamethoxazole and erythromycin; and 42.9% for neomycin, chloramphenicol and norfloxacin. In conclusion, the chicken meat and giblets were tainted by *H. pylori*, with a higher occurrence of the *ureC*, *babA2*, *vacA* and *cagA* genotypes. Future investigations should investigate the resistance of *H. pylori* to various antimicrobial agents in Egypt.

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## 1. Introduction

Chickens are an important economic source of animal protein for humans (Ammar et al., 2015; Hussain et al., 2015). In restricted slaughtering facilities, chickens are slaughtered, plucked and commonly eviscerated by hand. During evisceration, the carcass is evacuated, the visceral organs are removed, and the liver, heart and gut are collected (Ammar et al., 2015). These organs might be tainted by the spillage of the intestinal contents. After evisceration, the carcasses are washed with water, which may be a primary source of microbial contamination (Arnold, 2007). Millions of people consume chicken daily as a source of animal protein worldwide; thus, hygienic methods of supplying chickens for consumption are extremely relevant to public health.

Helicobacter species are gram-negative, microaerophilic spiral bacterial pathogens that can be exceedingly pathogenic and have been observed to settle in the biliary tract and gut in various animals. According to their favored site of colonization, these

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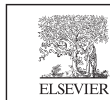


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organisms are classified as gastric or enterohepatic Helicobacter (Stanley et al., 1994). These two groups are considered zoonotic microorganisms (Josenhans et al., 2000). In general, during infection by the gastric Helicobacter group, the bacteria colonize the stomach; the enterohepatic Helicobacter group principally colonizes the distal portion of the digestive system and biliary duct (Hassan et al., 2014). *Helicobacter pullorum (H. pullorum)*, which was first isolated from the cecum of apparently healthy domestic fowls and the small intestine and liver of fowls with severe gastroenteritis and hepatitis (Stanley et al., 1994; Zanoni et al., 2007; Qumar et al., 2017), is a member of the enterohepatic Helicobacter group. Furthermore, *Helicobacter hepaticus* (*H. hepaticus*), *Helicobacter canis* (*H. canis*), *Helicobacter bilis* (*H. bilis*) and *Helicobacter cinaedi* (*H. cinaedi*) were isolated from chickens (Taylor et al., 2003; Young et al., 2004).

*Helicobacter pylori* (*H. pylori*) is a major human pathogenic bacterium associated with stomach cancer and duodenal ulcers (Wong et al., 2004; El Dairouty et al., 2016). Data regarding the prevalence and spread of infection caused by *H. pylori* are essential to control its spread and distinguish high-risk individuals, particularly in districts with an unexpected incidence of gastritis and stomach cancer (Safaei et al., 2011; Rahimi and Kheirabadi, 2012; Momtaz et al., 2014; Mousavi et al., 2014). Although *H. pylori* strains have been previously isolated from various food products, the significant role of foods of animal origin in the spread of *H. pylori* infection remains unclear (El Dairouty et al., 2016; Hemmatinezhad et al., 2016).

The pathogenicity of *H. pylori* is related to virulence factors. Sicinschi et al. (2008) showed that *H. pylori* is genetically mutable, and certain virulence genes are only identified in certain populations. Multiplex polymerase chain reaction (Multiplex-PCR) has been used to identify *H. pylori* isolated from various medical samples. Numerous virulence genes in *H. pylori* strains, such as the urease C (*ureC*), cytotoxin-associated A (*cagA*) and vacuolating cytotoxin (*vacA*) genes, have been identified and may play a role in the development of infection caused by *H. pylori* (Erzin et al., 2006). The *ureC* gene of *H. pylori* encodes a phosphoglucosamine mutase that was recently renamed *glmM*. De Reuse et al. (1997) considered this gene a "housekeeping" gene that contributes to the development and growth of the bacterial cell wall. The *cagA* gene is present in approximately 50% of all isolates of *H. pylori* and is responsible for inflammation in the gastric mucosa, the production of interleukin-8 (IL-8) and the pathogenesis of gastric cancer (van der Ende et al., 1998). In addition, Chomvarin et al. (2008) demonstrated that the *vacA* gene is found in all isolates of *H. pylori* and is responsible for the pathogenesis of stomach carcinoma and ulcers by damaging the gastric mucosa. The *babA2* gene is a membrane protein in *H. pylori* that contributes to the binding activity to the gastric mucosa (Pride et al., 2001). Consequently, the molecular genotyping of *H. pylori* using Multiplex-PCR is considered an intensive method for determining its pathogenicity.

Due to the unexpected resistance of *H. pylori* against several antimicrobial agents, treatment is another significant strategy to prevent the spread of infection in the population (Mégraud, 2004). The resistance of *H. pylori* to various antimicrobial drugs differs by location and appears to be increasing over time in many regions (Meyer et al., 2002; De Francesco et al., 2010; Graham, 2015). Furthermore, using the multiple antibiotic resistance (MAR) index is considered an economic and effective method for bacterial source tracking. This index was previously investigated by Krumperman (1983), who reported that an index of 0.2 indicates a higher incidence of infection where antimicrobial agents are frequently used. To date, no studies investigating the antimicrobial resistance of *H. pylori* isolated from edible and non-edible chicken organs in Egypt have been published. The significance of *H. pylori* and the epidemiology of this pathogenic bacteria in Egypt remain unclear. Animal-derived foods, particularly chicken, should be considered to prevent and control *H. pylori* infection in humans. Therefore, the current study examined *in vitro* the spread of the *glmM*, *babA2*, *vacA* and *cagA* virulence genotypes and their resistance to various antibiotics in *H. pylori* strains isolated from the meat and giblets of broiler chickens.

## 2. Materials and methods

### 2.1. Sample origin

Ninety chicken specimens, including meat (n = 30), gizzard (n = 30) and liver (n = 30) samples, were collected from a semi-automatic abattoir in Sadat City, Menoufia Governorate, Egypt, and were examined in this study. Each specimen was placed in a special water-resistant sterilized plastic bag. The specimens were obtained from the meat, livers and gizzards, including the jejunum, cecum and colon, for isolation and molecular identification by Multiplex-PCR. All specimens were kept at −80 °C until further investigation.

### 2.2. Identification of Helicobacter species

#### 2.2.1. Colony morphology and gram staining

Typical colonies of *Helicobacter* incubated for 5–7 days on *Helicobacter Pylori* Special Peptone (HPSP) agar medium appeared as clear, circular colonies with a diameter of 0.5–2 mm. These colonies were transferred to slants and gram-stained to visualize the gram-negative, S- or C-shaped organisms. Rod and coccoid shapes were observed. The pure colonies were subjected to further identification using biochemical, molecular and antibiotic sensitivity tests. The *H. pylori* ATCC ® 43,504 strain was utilized in the current investigation as a reference strain.

#### 2.2.2. Biochemical analysis of H. Pylori by urease, oxidase and catalase tests

To rapidly identify *H. pylori,* the urease test was performed according to the method previously described by MacFaddin (2000). A pure culture of the tested organism was streaked onto the whole surface of a urea agar plate. The test tubes were incubated at 37 °C in ambient air for 18–24 h. The oxidase test was also performed to biochemically identify *H. pylori*, which produces cytochrome oxidase enzyme. The oxidase activity (blue/purple color) was evaluated in all isolates using oxidase test strips (Sigma-Aldrich, USA). Moreover, the catalase activity in the isolated strains was examined using the drop technique. In brief, hydrogen peroxide ($H_2O_2$) was added to a pure colony, which was then directly transferred to a slide. The formation of oxygen bubbles was considered a positive result.

#### 2.2.3. Genotypical identification of H. Pylori by Multiplex-PCR

*2.2.3.1. Primer sequences.* 16S rRNA was applied to detect the Helicobacter species. The *UreC* (*glmM*), *babA2*, *cagA* and *vacA* genes were utilized to molecularly characterize *H. pylori*. All oligonucleotide sequences were designated by Pharmacia Biotech Company (Table 1).

*2.2.3.2. DNA extraction.* The *H. pylori* DNA was extracted as described by Shah et al. (2009). Briefly, 2 or 3 colonies of overnight culture were inoculated in micro-centrifuge tubes containing 120 µl of phosphate buffer saline (PBS) and mixed carefully for 2 min. All tubes were boiled for 15 min at 100 °C, cooled and centrifuged

**Table 1**
Oligonucleotide sequences, product length and cycling conditions of *H. pylori* virulence genotypes.

| Target gene | Oligonucleotide sequence (5′ → 3′) | bp | Initial denaturation | Amplification (35 cycles) | | | Final extension | Reference |
|---|---|---|---|---|---|---|---|---|
| | | | | Secondary denaturation | Annealing | Extension | | |
| *16S rRNA* | CTATGACGGGTATCCGGC<br>ATTCCACCTACCTCTCCCA | 375 | 94 °C<br>5 min | 94 °C<br>30 s | 53 °C<br>60 s | 72 °C<br>90 s | 72 °C<br>5 min | Riley et al. (1996) |
| *UreC* | GAATAAGCTTTTAGGGGTGTTAGGGG<br>GCTTACTTTCTAACACTAACGCGC | 294 | 94 °C<br>10 min | 94 °C<br>1 min | 51 °C<br>1 min | 72 °C<br>1 min | 72 °C<br>10 min | Safaei et al. (2011) |
| *BabA2* | ATGGAAATACAACAAACACAC<br>CTGCTTGAATGCGCCAAAC | 259 | 94 °C<br>3 min | 94 °C<br>1 min | 55 °C<br>1 min | 72 °C<br>1 min | 72 °C<br>10 min | Paniagua et al. (2009) |
| *CagA* | CAATCTGTCCAATCAAGCGAG<br>GCGTCAAAATAATTCCAAGG | 350 | 94 °C<br>3 min | 94 °C<br>1 min | 55 °C<br>1 min | 72 °C<br>1 min | 72 °C<br>10 min | Chattopadhyay et al. (2004) |
| *VacA* | GTTGATAACGCTGTCGCTTC<br>GGGTTGTATGATATTTTCCATAA | 567 | 94 °C<br>3 min | 94 °C<br>1 min | 63 °C<br>1 min | 72 °C<br>1 min | 72 °C<br>10 min | Chattopadhyay et al. (2004) |

at 13,000-×g for 1 min. Finally, 200 μl of the extract were inoculated in a sterile microcentrifuge tube and frozen at −20 °C until use.

*2.2.3.3. Amplification reactions of the 16S rRNA, ureC, babA2, cagA and vacA genes.* The amplification of the 16S rRNA was performed as described by Moyaert et al. (2008). The total volume of 25 μl consisted of 5 μl of deoxynucleoside triphosphate mix, 2.5 μl of 10 × PCR buffer, 0.25 μl of the primer, and 1 μl of the DNA template. The amplification of the *ureC* gene was performed according to the method described by Kianpour et al. (2014). The total volume of 50 μl consisted of 5 μl 10 × buffer + MgCl2, 2 mM dNTP, 2 unit Taq DNA polymerase, 100 ng DNA template, and 25 pmol (pmol) of each primer. The amplification of the *babA2*, *cagA* and *vacA* virulence genes was performed as previously described by Paniagua et al. (2009). In brief, a total volume of 25 μl (2.5 pmol of *babA2*-F and *babA2*-R, 25 pmol of *vacA*-F and *vacA* -R, 10 pmol of *cag5c*-F and *cag3c*-R, 0.25 mM of each dNTP, 0.9 U of Taq DNA polymerase and 1.5 mM of MgCl2) was applied. Taq polymerase, $MgCl_2$, and nuclease-free water were utilized appropriately in each test. The amplification of all genes was performed using a Thermal Cycler (Master cycler, Eppendorf, Hamburg, Germany). The amplifications were performed as shown in Table 1. Finally, the amplified DNA fragments of all genes were investigated using 2% agarose gel electrophoresis (AppliChem, Germany, GmbH) in 5 μl/100 ml Tris-borate-EDTA (TBE) buffer stained with ethidium bromide and visualized using an ultra-violet (UV) transilluminator.

### 2.3. Antibiotic sensitivity and MAR index of H. Pylori

Antibiotic discs (Oxoid Limited, Basingstoke, UK) with variable concentrations were utilized in the present investigation to determine the *in vitro* sensitivity of *H. pylori* strains to 14 antimicrobial agents commonly used to treat *H. pylori*. The antibiotic discs used in our investigation were streptomycin (S), 25 μg; amoxicillin (AMX), 10 μg; penicillin (P), 10 U; oxytetracycline (T), 30 μg; nalidixic acid (NA), 30 μg ampicillin (AM), 25 μg; sulfamethoxazole (SXT), 100 μg; erythromycin (E), 15 μg; neomycin (N), 30 μg; chloramphenicol (C), 50 μg, norfloxacin (NOR), 10 μg; kanamycin (K), 5 μg; ciprofloxacin (CP), 10 μg and gentamycin (G), 200 μg. The results were interpreted as susceptible, intermediate, or resistant according to the zone diameter interpretative standards suggested by the Clinical and Laboratory Standards Institute (CLSI, 2001). The MAR index of each strain was also detected using the equation provided by Singh et al. (2010) as follows:

$$\text{MAR index} = \frac{\text{Number of antimicrobial drugs to which the bacterium is resistant}}{\text{Total number of antimicrobial drugs}}$$

## 3. Results

### 3.1. Incidence of Helicobacter species in broiler chickens

The incidence of *H. pylori* was investigated in ninety chicken meat, gizzard and liver samples. The frequency of the Helicobacter species in the chicken meat and giblets is shown in Table 2. According to the results, of the 90 broiler chicken samples, 7 (7.78%), 4 (4.44%), 2 (2.22%), 2 (2.22%) and 1 (1.11%) samples were positive for *H. pylori*, *H. pullorum*, *H. cinaedi, H. bilis* and *H. hepaticus*, respectively. Among the samples positive for *H. pylori*, 3 of the 7 (42.86%) isolates were isolated from the liver, and the other 4 isolates were isolated from both the meat and gizzard.

### 3.2. Biochemical analysis of H. Pylori strains

A rapid biochemical identification of the *H. pylori* strains was performed using urease, oxidase and catalase tests. After 4 h of incubation, the 7 positive *H. pylori* strains were identified by a purple color, a blue/purple color and the formation of oxygen bubbles by the urease, oxidase and catalase tests, respectively.

### 3.3. Molecular identification of H. Pylori virulence genes

Fig. 1 illustrates the electrophoresis results of the PCR products of the 16 Helicobacter species isolated from the 90 chicken liver, meat and gizzard samples. The 375-bp PCR product of 16S rRNA was detected in 15/16 (93.75%) of the *Helicobacter* species and identified as 7 (43.75%) *H. pylori*, 4 (25%) *H. pullorum*, 2 (12.5%) *H. cinaedi*, 1 (6.25%) *H. hepaticus* and 1 (6.25%) *H. bilis* strains. To characterize *H. pylori*, the 294-bp PCR product representing the presence of the *ureC* gene was detected in all *H. pylori* strains (Fig. 2). The frequency of the *babA2*, *cagA* and *vacA* genes in the *H. pylori* strains is demonstrated in Fig. 3 and Table 3. Of the 7 *H. pylori* strains, the percentages of these three genes were 7/7 (100%), 4/7 (57.1%) and 3/7 (42.9%). Thus, the most common virulence factor in *H. pylori* was the *babA2* gene. In addition, the isolates from the chicken liver had the highest frequency of the tested virulence genes.

### 3.4. Antimicrobial susceptibility and MAR index of H. Pylori

As revealed in Table 4, the resistance of the *H. pylori* strains was 100% against streptomycin, 85.7% against amoxicillin and penicillin, 71.4% against oxytetracycline, nalidixic acid and ampicillin, 57.1% against sulfamethoxazole and erythromycin, and 42.9% against neomycin, chloramphenicol and norfloxacin. In contrast, the lower resistance rates were 14.3% against gentamycin and 28.6% against ciprofloxacin and kanamycin. Table 5 demonstrates

**Table 2**
Incidence of *Helicobacter* species isolated from examined samples of chicken meat and giblets.

| Helicobacter species | Chicken meat | | Chicken gizzard | | Chicken liver | | Total (90) | |
|---|---|---|---|---|---|---|---|---|
| | No. | % | No. | % | No. | % | No. | % |
| *H. pylori* | 2 | 6.67 | 2 | 6.67 | 3 | 10.00 | 7 | 7.78 |
| *H. pullorum* | 1 | 3.33 | 2 | 6.67 | 1 | 3.33 | 4 | 4.44 |
| *H. cinaedi* | 1 | 3.33 | 1 | 3.33 | 0 | 0 | 2 | 2.22 |
| *H. bilis* | 0 | 0 | 0 | 0 | 2 | 6.67 | 2 | 2.22 |
| *H. hepaticus* | 0 | 0 | 0 | 0 | 1 | 3.33 | 1 | 1.11 |
| Total | 4 | 13.33 | 5 | 16.67 | 7 | 23.33 | 16 | 17.78 |

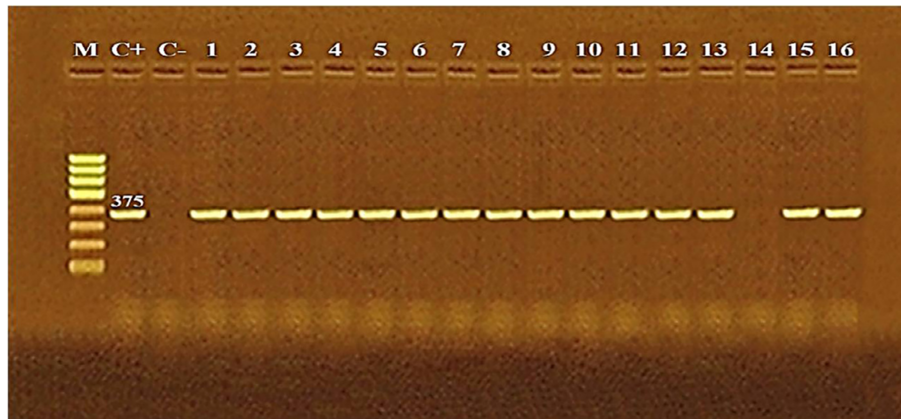


**Fig. 1.** Agarose gel electrophoresis of PCR amplification products using 16S rRNA (375 bp) as a specific primer to identify the Helicobacter species. Lane M: 100-bp ladder as molecular DNA marker; lane C+: positive control for 16S rRNA of Helicobacter species; lane C−: negative control. Lanes 1–7: positive *H. pylori*; lanes 8–11: positive *H. pullorum*; lanes 12–13: positive *H. cinaedi*; lane 15: positive *H. bilis*; lane 16: positive *H. hepaticus;* and lane 14: negative Helicobacter species.

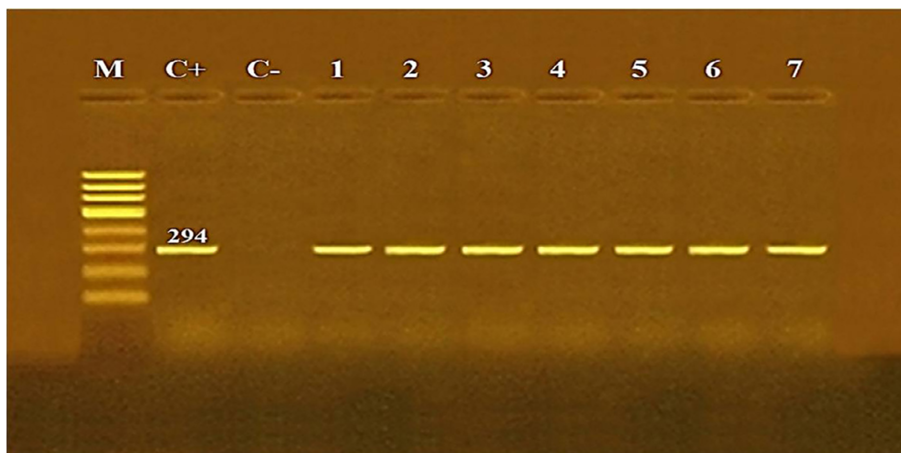


**Fig. 2.** Agarose gel electrophoresis of PCR of the *ureC* gene (294 bp) for the characterization of the *H. pylori* strains. Lane M: 100-bp ladder as molecular size DNA marker; lane C+: positive control *H. pylori* for *ureC* gene; lane C−: negative control; and lanes 1–7: positive *H. pylori* strains.

the MAR index of 7*H. pylori* strains in the chicken meat and giblets. The average MAR index of all *H. pylori* strains was 0.571. Six of the seven *H. pylori* strains revealed resistance against various antimicrobial drugs with a MAR index ranging from 0.21–1.0. Strain No. 1 showed strong resistance against all antimicrobial agents (MAR index of 1.0), and strain No. 2 demonstrated resistance against 13 of the 14 antibiotics (MAR index of 0.92). For strain Nos. 3–6, the MAR index values were 0.78, 0.57, 0.42 and 0.21. The proportion of *H. pylori* strains with a MAR index > 0.2 was 5/7 (71.42%); the proportion of strains with a MAR index $\leq$ 0.2 was 2/7 (28.57%). Thus, *H. pylori* is highly resistant against many tested antimicrobial drugs with high MAR index values.

## 4. Discussion

To date, evidence implicating chickens as an important reservoir of the *H. pylori* found in humans is lacking. Therefore, we suggest that the *H. pylori* strains isolated from the broiler chicken meat, livers and gizzards in the present study were obtained during slaughtering and/or processing. Because humans are considered the natural reservoir of this bacterium, the abattoir workers were likely the main source of the *H. pylori* infection in our chicken samples. In the current investigation, 7 (7.78%) isolates of *H. pylori* were found in the 90 broiler chicken samples, suggesting that this bacterium represents a risk to human beings. Nevertheless, the

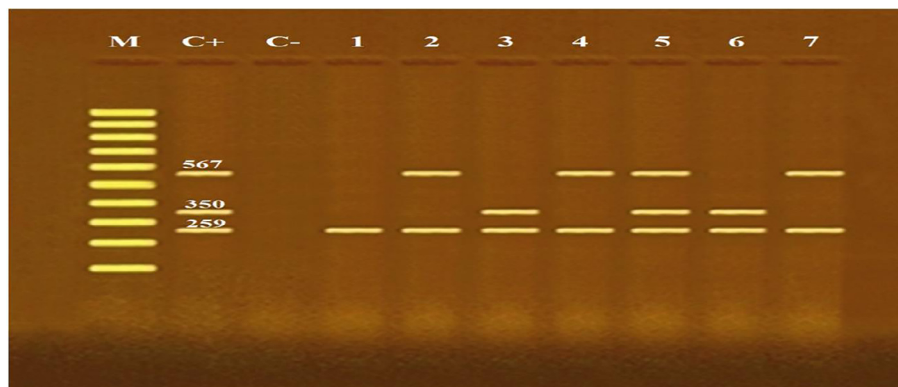


**Fig. 3.** Agarose gel electrophoresis of Multiplex-PCR of *babA2* (259 bp), *cagA* (350 bp) and *vacA* (567 bp) as virulence genes of *H. pylori* strains. Lane M: 100-bp ladder as molecular size DNA marker; lane C+: positive control strain for the *babA2*, *cagA* and *vacA* genes; lane C−: negative control. Lane 1: positive *H. pylori* strain for the *babA2* gene. Lanes 2, 4 and 7: positive *H. pylori* strains for the *babA2* and *vacA* genes. Lanes 3 and 6: positive *H. pylori* strains for the *babA2* and *cagA* genes. Lane 5: positive *H. pylori* strain for the *babA2*, *cagA* and *vacA* genes.

**Table 3**
Incidence of *babA2*, *vacA* and *cagA* genes as virulence factors in isolated *H. pylori* using Multiplex-PCR.

| Virulence genes | Chicken meat (2) | | Chicken gizzard (2) | | Chicken liver (3) | | Total (7) | |
|---|---|---|---|---|---|---|---|---|
| | No. | % | No. | % | No. | % | No. | % |
| *BabA2* | 2 | 100 | 2 | 100 | 3 | 100 | 7 | 100 |
| *VacA* | 1 | 50 | 1 | 50 | 2 | 66.7 | 4 | 57.1 |
| *CagA* | 0 | 0 | 1 | 50 | 2 | 66.7 | 3 | 42.9 |

**Table 4**
Percentages of *H. pylori* antimicrobial resistance (n = 7).

| Antimicrobial agent | S | | I | | R | |
|---|---|---|---|---|---|---|
| | NO | % | NO | % | NO | % |
| Streptomycin (S) | – | – | – | – | 7 | 100 |
| Amoxicillin (AMX) | – | – | 1 | 14.3 | 6 | 85.7 |
| Penicillin (P) | 1 | 14.3 | – | – | 6 | 85.7 |
| Oxytetracycline (T) | – | – | 2 | 28.6 | 5 | 71.4 |
| Nalidixic acid (NA) | 1 | 14.3 | 1 | 14.3 | 5 | 71.4 |
| Ampicillin (AM) | 1 | 14.3 | 1 | 14.3 | 5 | 71.4 |
| Sulfamethoxazole (SXT) | – | – | 3 | 42.9 | 4 | 57.1 |
| Erythromycin (E) | 2 | 28.6 | 1 | 14.3 | 4 | 57.1 |
| Neomycin (N) | 2 | 28.6 | 2 | 28.6 | 3 | 42.9 |
| Chloramphenicol (C) | 3 | 42.9 | 1 | 14.3 | 3 | 42.9 |
| Norfloxacin (NOR) | 2 | 28.6 | 3 | 42.9 | 3 | 42.9 |
| Kanamycin (K) | 4 | 57.1 | 1 | 14.3 | 2 | 28.6 |
| Ciprofloxacin (CP) | 5 | 71.4 | 1 | 14.3 | 2 | 28.6 |
| Gentamycin (G) | 6 | 85.7 | – | – | 1 | 14.3 |

**Table 5**
Antimicrobial resistance profile of *H. pylori strains* (n = 7).

| No. | Antimicrobial resistance profile | MAR index |
|---|---|---|
| 1 | S, AMX, P, T, NA, AM, SXT, E, N, C, NOR, K, CP, G | 1 |
| 2 | S, AMX, P, T, NA, AM, SXT, E, N, C, NOR, K, CP | 0.928 |
| 3 | S, AMX, P, T, NA, AM, SXT, E, N, C, NOR | 0.786 |
| 4 | S, AMX, P, T, NA, AM, SXT, E | 0.571 |
| 5 | S, AMX, P, T, NA, AM | 0.429 |
| 6 | S, AMX, P | 0.214 |
| 7 | S | 0.071 |
| Average 0.571 | | |

E: Erythromycin, NA: Nalidixic acid, P: Penicillin, AMX: Amoxicillin, T: Oxytetracycline, SXT: Sulfamethoxazole, AM: Ampicillin, S: Streptomycin, N: Neomycin, C: Chloramphenicol, NOR: Norfloxacin, CP: Ciprofloxacin, K: Kanamycin, G: Gentamycin.

core cause underlying this finding is unclear, but cross-contamination of chicken carcasses appears to be a principal cause of *H. pylori* incidence in poultry slaughterhouses. Processing, storing and transporting chicken carcasses are the main three processes that may increase the frequency of *H. pylori* contamination. Another investigation conducted by Ranjbar et al. (2016) demonstrated that *H. pylori* can live in water. Therefore, using contaminated water in slaughterhouses is considered another reason for the presence of *H. pylori* in the chicken samples. Moreover, infected hand workers and slaughterhouse equipment, such as knives, may also cause the higher incidence of this bacterium (Gilani et al., 2017). In general, our results are consistent with those obtained by Meng et al. (2008), who tested 11 raw chicken samples (whole chicken with skin) by Multiplex-PCR and found that 4 (36%) samples were *H. pylori*-positive, but our percentages were much lower. In addition, these authors indicated that *H. pylori* is considered a foodborne pathogen that can be transmitted to consumers. El Dairouty et al. (2016) investigated the incidence of *H. pylori* in 30 raw meat, 20 raw poultry and 20 luncheon meat samples and found that 5% of each were *H. pylori* positive.

Recently, numerous researchers have used molecular methods to detect the different genotypes of *H. pylori*, which are closely

related to its epidemiology (Suerbaum and Josenhans, 2007). Generally, Multiplex-PCR is used as an assay for the genotyping and identification of conserved genes in *H. pylori* strains isolated from clinical samples (Lu et al., 1999; Espinoza et al., 2011). In the current study, the 16S rRNA and *ureC* genes were used as housekeeping genes. The distribution of these genes was 93.75% and 100%, suggesting that the *ureC* gene is a suitable gene for the identification of various strains of *H. pylori*. Lu et al. (1999) and El Dairouty et al. (2016) obtained similar results. These authors clarified that compared with the 16S rRNA gene, the *ureC* gene is a specific gene for the recognition of *H. pylori* strains isolated from stomach samples. The *ureC* gene is very important for the growth and cell wall development of *H. pylori*. Hence, this gene has been extensively utilized for the identification of *H. pylori* (Kusters et al., 2006). The distribution of the *babA2*, *vacA* and *cagA* virulence genes was also investigated in our study. The *babA2* (100%), *vacA* (57.1%) and *cagA* (42.9%) genes were commonly identified in the *H. pylori* strains obtained from the edible and non-edible organs from the chicken slaughterhouses. Thus, these virulence genotypes, particularly *babA2*, had a higher incidence in the liver and meat of broiler chickens, which are considered ready to eat food samples for humans. These genotypes likely increase the destructive effect of *H. pylori* in the human stomach (Biernat et al., 2014). The potential link between the presence of the *H. pylori* *babA2/cagA+/vacAs1* genotypes and the frequency of gastritis, gastric cancer and duodenal ulcers was previously proposed by Gerhard et al. (1999) and Bibi et al. (2017).

The high incidence of antimicrobial resistance among the *H. pylori* strains is another significant finding in the current study. Various antibiotics were tested in our investigation, and *H. pylori* strains exhibited strong resistance to streptomycin, amoxicillin, penicillin, oxytetracycline, nalidixic acid, ampicillin, sulphamethoxazol, erythromycin, neomycin, chloramphenicol and norfloxacin. Mousavi et al. (2014) obtained similar results. These authors found that *H. pylori* strains in milk showed strong resistance against ampicillin (84.4%), tetracycline (76.6%), erythromycin (70.5%) and metronidazole (70%). Moreover, former studies reported by Thyagarajan et al. (2003), Secka et al. (2013) and Yahaghi et al. (2014) indicated that *H. pylori* in food specimens had extreme rates of resistance against amoxicillin, metronidazole, ampicillin and oxytetracycline. Moreover, epidemiological surveys conducted in China, Taiwan, the Kingdom of Saudi Arabia, Egypt, Nigeria, Iran, India, Brazil, Argentina and Colombia indicated that *H. pylori* strains in medical samples had a potent degree of resistance against various antimicrobial agents, such as amoxicillin, metronidazole, quinolones and tetracycline (WGO, 2010), which is consistent with our findings. According to the analysis of the MAR index, 71.42% of the *H. pylori* strains demonstrated strong resistance against three or more of the antimicrobial drugs used in the current investigation, indicating a high risk of contamination in chickens. The increased frequency of antimicrobial resistance in our investigation might be due to the unselective use of these antimicrobial drugs. Many reports have investigated the resistance rate of *H. pylori* against various antibiotics, but several studies have had difficulties, particularly regarding the number of tested strains (Mégraud, 2004). Our detection of antibiotic resistance showed that *H. pylori* in the meat, livers and gizzards were transferred from infected poultry samples. The lower resistance of the *H. pylori* strains to gentamycin, ciprofloxacin and kanamycin was also revealed in our study, which may be due to the less frequent prescription of these antimicrobial drugs.

## 5. Conclusions

In Egypt, chicken meat and giblets are tainted by *H. pylori* with the *glmM*, *babA2*, *vacA* and *cagA* virulence genes. Thus, raw and/or uncooked chicken meat and liver might be a source of *H. pylori* infection in human beings. The most significant finding in our investigation is that chickens are another source harboring virulent strains of *H. pylori*. Thus, good hygienic practices for abattoirs and butchers play a vital role in decreasing the danger of spreading *H. pylori* from chicken meat and giblets to people. Moreover, the *H. pylori* strains demonstrated high resistance against streptomycin, amoxicillin, penicillin, oxytetracycline, nalidixic acid, ampicillin, sulfamethoxazole, erythromycin, neomycin, chloramphenicol and norfloxacin, with high multiple antibiotic index values. In contrast, *H. pylori* had low resistance against gentamycin, ciprofloxacin and kanamycin; therefore, we recommend using these antimicrobial agents for the treatment of *H. pylori* in Egypt.

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