# Isolation and identification of *Helicobacter pylori* from raw chicken meat in Dhamar Governorate, Yemen

**Dhary Alewy Almashhadany,[1] Sara Mohammed Mayas,[2] Noor Lutphy Ali[3]**

**[1]Department of Medical Laboratory Science, College of Science, Knowledge University, Erbil, Iraq; [2]Department of Microbiology, Faculty of Applied Science, Thamar University, Yemen;[3]Department of Medical Microbiology, College of Science, Cihan University-Erbil, Kurdistan Region, Iraq**

## Abstract

Although *Helicobacter pylori* (*H. pylori*) is one of the most common bacterial pathogens of human, its natural reservoirs are still unclear. There is an increasing number of reports that document the occurrence of *H. pylori* in various foods. This study aimed at isolation of *H. pylori* from chicken meat sampled. Two hundred and sixty samples were collected randomly from slaughterhouses and markets in Dhamar Governorate, Yemen. Samples were enriched in Brain-Heart Infusion broth in microaerophilic conditions before inoculating the Camp-Blood agar and EYE agar plates. Results showed that 13.8% of samples were contaminated evidenced by *H. pylori* growth via traditional culture method on agar media. No significant differences between sample types (thighs and breast muscles) (p=0.353) or the sampling source (p=0.816) were observed. Autumn season was associated with increased occurrence of *H. pylori*. The source of *H. pylori* in food is still not identified. Proper cooking and good sanitation practices are highly recommended to avoid the infection. Further studies addressing the potential sources of *H. pylori* are highly suggested.

## Introduction

*Helicobacter pylori* (*H. pylori*) is one of the most prevalent human bacterial pathogens globally (Sjomina *et al.,* 2018). It is estimated that about two-thirds of the world's population are infected with *H. pylori*, predominantly in developing countries with higher occurrence in poor and unhygienic areas. The prevalence of *H. pylori* infection depends on diverse contributing factors such as socioeconomic status, geographical area, living conditions, and personal hygiene (Sjomina *et al.,* 2018; Al Mashhadany, 2020). Infected individuals are the main reservoir of *H. pylori*, however, most of these infections are asymptomatic (Denic *et al.,* 2020). Clinically, *H. pylori* infection in human is associated with chronic gastritis, peptic ulceration, duodenal ulcer, gastric cancer as well as mucosa associated lymphoid malignancies (Almashhadany & Mayass, 2018; Denic *et al.,* 2020).

From bacteriology perspective, *H. pylori* is ~2-3.5×0.5-1.0 µm small, curved, microaerophilic, lophotrichous gram-negative, S-shaped or curved rod bacterium. It has copious amounts of urease enzyme to survive the acidic environment of the stomach by converting urea to ammonia. The production of ammonia around *H. pylori* neutralizes the acidity of the stomach, making it more hospitable for *H. pylori.* Moreover, the helical shape of *H. pylori* allows it to be hidden in the mucus layer which is less acidic than the surface or the lumen of the stomach (Saeidi & Sheikhshahrokh, 2016; Al-Mashhadany *et al.,* 2018).

Molecular epidemiology studies had detected *H. pylori* DNA in different foodstuffs, water, and animals which suggest the existence of reservoirs for *H. pylori* outside human gastrointestinal tract (Momtaz *et al.,* 2014; Mousavi *et al.,* 2015). Milk, meat, and vegetables are a potential source of *H. pylori* infections (Duynhoven & Jonge, 2001; Herrera, 2004). Milk products are the most studied, probably because the infection is mainly acquired during childhood and milk is mostly consumed during this period (Al-Mashhadany & Mayass, 2017; Talimkhani & Mashak, 2017). Nonetheless, the role of foods as a transmission medium is not well-validated clinically. The most commonly accepted hypothesis of *H. pylori* transmission is the oral-fecal route (Sjomina *et al.,* 2018). Despite the absence of solid evidence of foods as a reservoirs for *H. pylori*, different studies had isolated or identified different strains and raised concerns about the contribution of nonhuman sources (Keenan *et al.,* 2010; Talimkhani & Mashak, 2017; Hamada *et al.* 2018). Suboptimal sanitation conditions are favored for oral-fecal & oral-oral transmission of *H. pylori* in institutions of disable individuals and orphanages (Sjomina *et al.,* 2018). Several studies have reported the survival and presence of *H. pylori* in foods and water, particularly in ready-to-eat products and milk, proposing that they can be sources of infection (Quaglia & Dambrosio, 2018). Foods intrinsic factors, such as pH ranging (4.9 to 6.0) and water activity (>0.97) could theoretically provide good conditions for *H. pylori* survival. Therefore, data on survival ability may be more significant than concerns about the growth of the bacteria in foods when determining the role of different types of food in *H. pylori* transmission to humans (Quaglia *et al.,* 2007; Quaglia & Dambrosio, 2018; Al-mashhadany 2018). The prevalence of *H. pylori* infection in Yemen is not well-defined as various studies reported a wide range of 10-82.2% (Gunaid *et al.* 2003, Al-Shamahy 2005, Bahumid *et al*. 2009, Almashhadany & Mayass 2018), and its transmission routes in poor developing countries are a matter for wide debated (Alsulaimany *et al.* 2020). This study was conducted to detect the occurrence of *H. pylori* in raw chicken meat at Dhamar Governorate (Yemen). The relationship between occurrence of *H. pylori* in chicken meat and months during the period of study was also addressed.

Correspondence: Dhary Alewy Almashhadany, Department of Medical Laboratory Science, College of Science, Knowledge University, Erbil 44001, Iraq.
Tel.: +9647733565479.
E-mail: Dhary.alewy@knu.edu.iq

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## Materials and Methods

### Study design and sampling

Two hundred and sixty (260) fresh raw chicken meat samples (140 Thigh, and 120 Breast), were collected from retail markets and chicken slaughterhouses in different places of Dhamar Governorate, from July to December 2020. The samples were put in sterile cooled polyethylene bags and kept in ice box with temperature approximately 4°C during transport and storage at the laboratory (Almashhadany, 2021b). The bacteriological analysis was performed within 2 h of sample collection.

### Isolation of *H. pylori*

In the laboratory, the isolation of *H. pylori* was done under aseptic conditions as previously published (Al-Mashhadany & Mayass, 2017; Almashhadany, 2021a). Briefly, samples were cut into small pieces using sterile blades for liberation of adherent bacteria. From thigh and breast, 25 gm (as an optimal sample size) was soaked in 250 ml of normal saline. For enrichment, a volume of 0.5 ml of the suspension was then placed in a 4.5-ml Brain-Heart Infusion broth with 7% horse serum without antibiotics and incubated in a microaerophilic atmosphere (GasPack; Oxoid, Basingstoke, England) at 37°C for 3 to 7 days. After that, modified Campy-blood agar and EYE agar plates were inoculated with 100 µl of the enriched suspension and incubated at 37°C in microaerobic condition in a candle jar and Campy Gen (2.5 L) in the incubator for 4-10 days. For purification purposes, developed colonies were subcultures on the same agar media and incubated at 37°C for 48–72 hrs. (Coldham *et al.,* 2011; Lawson, 2015).

### Identification of *H. pylori*

Identification of *H pylori* isolates was done according to a published standard scheme (Lawson, 2015; Al-Mashhadany & Mayass, 2017). Briefly, after incubation, all cultural plates were examined for suspected colonies of *H. pylori.* Gram staining was done according to the standard protocol with exposure of smears to safranin for 3 minutes. Biochemical tests employed for the identification included: Catalase, Oxidase, Urease, Indole production, growth in 1% glycine, growth in 3.5% NaCl, $H_2S$ production in (TSI), TSI with lead acetate paper, resistance to nalidixic acid, sensitivity to cephalothin, and hippurate hydrolysis. Isolates that met the reference characteristics were considered *H. pylori* (Lawson, 2015; Al-Mashhadany & Mayass, 2017).

### Statistical analysis

Data were analyzed using SPSS software (version 25), confidence intervals (CI) were estimated using normal distribution approximation at an alpha level of 0.05. Chi-square test was used to evaluate differences between groups.

## Results

### Occurrence of *H. pylori* in raw chicken meat samples

From 260 raw chicken meat samples, 18 (13.8%) showed a positive result for *H. pylori*. This result includes 11 (15.7 %) positive samples from thigh and 7 (11.7%) positive samples from breast (Table 1). There is no significant difference between sample types in terms of contamination with *H. pylori* (p=0.353). Based on this sample size, up to 18% of chicken meat samples are expected to be contaminated with *H. pylori*.

### Occurrence of *H. pylori* according to sampling location

Regarding to the distribution of *H. pylori among* examined samples, the results showed a slightly higher occurrence of *H. pylori* in samples from slaughterhouses (Table 2). However, this increase was not significant statistically ($\chi^2$=0.054, *p*=0.816).

### Temporal distribution

The changes in occurrence of *H. pylori* were monitored throughout the study period. The highest rate of *H. pylori* was observed in October (23.8%) and September (22.7%), while the lowest rate was found in June (5.0%) and August (8.3%) (Figure 1). Autumn was significantly associated with increase in *H. pylori* contamination of chicken meat (p=0.007).

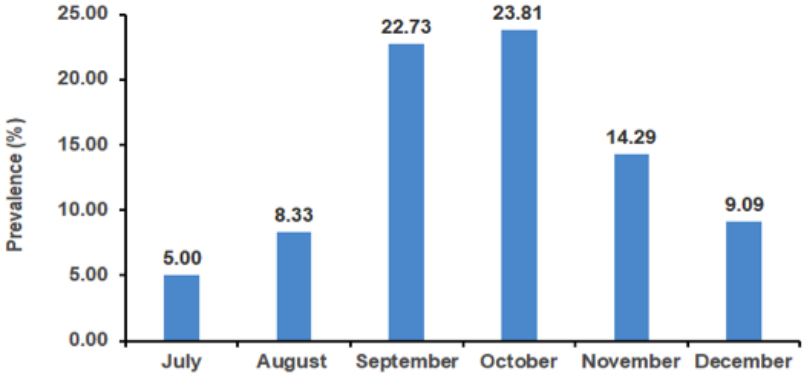


**Figure 1. Seasonal variations in occurrence of *H. pylori* during the period of study.**

**Table 1. Occurrence of *H. pylori* in raw chicken meat according to type of meat.**

| Chicken meat | No. samples | Positive Samples n (%) | 95% CI |
|---|---|---|---|
| Thigh | 140 | 22 (15.7) | 9.69 – 21.74 |
| Breast | 120 | 14 (11.7) | 5.92 – 17.41 |
| Total | 260 | 36 (13.8) | 9.65 – 18.04 |

**Table 2. Occurrence of *H. pylori* in raw chicken meat according to sampling location.**

| Chicken meat | Slaughterhouses | | Retail markets | |
|---|---|---|---|---|
| | No. of tested | Positive samples n (%) | No. of tested | Positive samples n (%) |
| Thigh | 80 | 12 (15.0) | 70 | 10 (14.3) |
| Breast | 60 | 8 (13.3) | 50 | 6 (12.0) |
| Total | 140 | 20 (14.3) | 120 | 16 (13.3) |

## Discussion

The occurrence and survival of *H. pylori* in different foods have been a hot area of research during the past decades. Studies addressing the occurrence of *H. pylori* in meat are rare, whereas the majority of published literature focused on milk and milk products (Herrera 2004, Quaglia & Dambrosio 2018). Recently, stomach of domestic animals have been found to harbor high numbers of *H. pylori*, which suggests domestic animals as an important reservoir (Saeidi & Sheikhshahrokh 2016). Therefore, this study aimed to detect *H. pylori* in chicken raw meat

Out of two hundred and sixty (260) raw chicken meat samples collected in this study, 36 (13.8%) were contaminated with *H. pylori*. This result is consistent with studies from Iran that found 10-14% of salad and vegetable samples harbored *H. pylori* (Atapoor *et al.* 2014, Yahaghi *et al.* 2014). Hemmatinezhad and associates in Iran, reported that 13.45% of ready-to-eat food samples were contaminated with *H. pylori* (Hemmatinezhad *et al.* 2016). Likewise, a similar occurrence was reported in raw milk detected by bacteriological culture or by molecular detection of *ureC* gene (Rahimi & Kheirabadi 2012, Kazemeini *et al.* 2014, Talaei *et al.* 2015). On the contrary, other studies documented higher rates (20 - 36%) in different foods including chicken raw meat (Dore *et al.* 2001, Meng *et al.* 2008, Mousavi *et al.* 2015, Saeidi & Sheikhshahrokh 2016). The role of food prepared under poor hygienic conditions as a possible vehicle for *H. pylori* transmission was suggested by Begue and colleagues, who found significant hazards for consumption of food obtained from street vendors in Peru (Begue *et al.* 1998). The actual sources of *H. pylori* in chicken raw meat have not been identified. However, contaminated water, infected handlers, and chicken gastrointestinal tract are the most probable sources (Meng *et al.* 2008, Vale & Vítor 2010, Quaglia & Dambrosio 2018).

Regarding the distribution of *H. pylori* among examined samples, it seems that sample type is not a contributing factor for occurrence of *H. pylori*. This observation is supported by a recent study in poultry slaughterhouses in Egypt that found 3.33% of liver samples to be contaminated with *H. pylori*, while 2.22% samples of meat and gizzard were contaminated (Hamada *et al.* 2018). According to our findings and previous investigations, *H. pylori* truly occur in foods (Duynhoven & Jonge 2001, Atapoor *et al.* 2014, Saeidi & Sheikhshahrokh 2016, Quaglia & Dambrosio 2018). However, techniques for direct isolation of *H. pylori* from foodstuff have not been fully developed or standardized. Indeed, the isolation of *H. pylori* from food products is quite difficult due to the presence of associated microflora and to the probably very low *H. pylori* load (Vale & Vítor 2010, Talimkhani & Mashak 2017).

The relationship between months and occurrence of *H. pylori* during the work in Dhamar Governorate was studied. The highest rates of isolation of *H. pylori* were found in October (23.8 %) and September (22.7 %). However, the occurrence of *H. pylori* was seen to decrease prior September and after October. This observation contradicts the previous study that did not find *H. pylori* in February, March, July, August, and September (Al-Mashhadany & Mayass 2017). In fact, the seasonality of *H. pylori* occurrence in poultry meat is still unaddressed. Autumn is a wet season in Dhamar that may provide favorable conditions for *H. pylori* proliferation in animals' gastrointestinal tracts that is reflected by higher contamination observed in September and October.

## Conclusions

*H. pylori* occurs in foods and may be an important media for its transmission (horizontal method). The occurrence of *H. pylori* in raw chicken meat in Dhamar Governorate seems to be high, mostly due to poor living conditions, socioeconomic status, and sanitary habits, or other risk factors. The seasonality of *H. pylori* in poultry meats is still unclear. In addition, special emphasis on proper cooking of chicken meat before consumption is recommended.

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