Pharmacy and Applied Health Science Journal
Volume 1, Number 1 (2022) Knowledge University

Isolation and Identification of Helicobacter Pylori from RTE Foods: Dhamar Governorate in Yemen as a Case Study

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Received: 08 November 2021
Accepted: 20 April 2022
Published: 30 June 2022
09 pages

How to cite this article: Almashhadany DA, Mayas SM. Isolation and Identification of Helicobacter Pylori from RTE Foods: Dhamar Governorate in Yemen as a Case Study. PHARM. APPL. H. SCI. [Internet]. 2022 Jun. 30;1(1):13-21. Available from: https://phahs.knu.edu.iq/index.php/phahs/article/view/5

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ABSTRACT

This study was designed to determine the prevalence of H. pylori in RTE foods at Dhamar Governorate from January until June 2021. Two hundred and eighty (280) samples were collected in this works; these samples included (85) chicken sandwiches, (80) Red meat sandwiches, and (115) Salad. Our result indicates that the total prevalence and isolation percentage of H. pylori from all collected samples was 37/280 (13.2%). The highest rate of pollution with H. pylori was found in Salad 17/115(14.8%), then in red meat sandwich 11/80(13.8%), while the lowest rate of occurrence was found in Chicken sandwich 9/85(10.6%). There is no significant difference between the isolation of Hp from different types of RTE foods and months. When we study the relationship between months and incidence of Hp during the research period, the results show that the highest rate of isolation of Hp from different kinds of RTE foods was found in April (18.6 %), approximately close to this rate in May (17.0 %). But the isolation rate was seen decrease whenever moved away from these three months and in both directions. There is no significant difference between the isolation of Hp from red meat and months ($p > 0.05$).

Kewords: Dhamar Governorate, Chicken sandwich, H.pylori, RTE foods, Yemen

1. Introduction

Helicobacter pylori (H.pylori) is one of the most prevalent human bacterial pathogens worldwide, with 4.4 billion infected individuals in 2015 [1]. It is estimated that about two-thirds of the world's population is infected with H.pylori, predominantly in developing countries with higher occurrence in poor and unclean areas. Generally, the overall prevalence is higher in underdeveloped regions, such as Africa and As more developed countries in Western Euro America. Overall, H.pylori prevalence is decreasing as a result of improved sanitary conditions and treatment procedures [2].

H.pylori infection varies from 7.3 % to 92.0 % depending on age, geographic location, the status of the populations [3]. Several studies have shown that the prevalence of H. pylori is still high in most countries. A higher incidence was reported in unclean and poor economic areas; the rate of H.pylori

infection in Africa, South America, and Asia was significantly higher than in Western Europe, North America, and Australia [4].

H.pylori is a gram-negative rod, microaerophilic, has a specific spiral shape,and is highly motile due to its multiple flagella that emerge from one rounded end.

From a bacteriology perspective, H.pylori is a small, curved, microaerophilic, lophotrichous gram-negative, ~ 2-3.5 µm × 0.5-1.0 µm, 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 stomach's acidity, making it more hospitable for H.pylori. Moreover, the spiral shape of H. pylori allows it to be hidden in the mucus layer, which is less acidic than the stomach's inside area, or lumen, [5, 6, 7, 8]. Over 80% of individuals infected with the bacterium are asymptomatic [4, 9], according to numerous studies that have confirmed the pathogenicity of H.pylori in gastritis, peptic ulcers, and gastric malignancies [10]. Recent studies have shown that eradicating H.pylori in infected individuals of all ages can reduce the occurrence of gastric cancers [11, 12].

H.pylori is one of the most prevalent human bacterial pathogens globally [13]. It is estimated that about two-thirds of the world's population is infected with H.pylori, predominantly in developing countries with higher occurrence in poor and unclean areas.

The prevalence of H.pylori depends on diverse contributing factors, including socioeconomic status, geographical, living conditions, and personal hygiene [13, 14]. Infected individuals are the main reservoir of H. pylori; however, most infections are asymptomatic [15]. H. pylori infection in humans is associated with chronic gastritis, peptic ulceration, duodenal ulcer, gastric cancer as well as mucosa-associated lymphoid malignancies [15, 16].

Several studies have shown that the prevalence of H. pylori is still high in most countries. In the south and east Europe, South America, and Asia, the prevalence of H.pylori is often higher than 50%. [17].

The prevalence of H.pylori is very high, according to previous studies. El-Gunaid et al., in 1991, found that there was a high prevalence of gastric H. pylori patients [18]. In addition, another showed that the oesophagal and gastric carcinoma accounted for as much as (6%) of all patients who had an upper gastrointestinal endoscopy over a year [19]. In 2003, Al-Shami showed that the prevalence of H.pylori infection among patients who underwent upper gastrointestinal endoscopy in Sana'a major hospital was very high (99.6 %) [20].

Gunaid et al. (2003) reported that the prevalence of H.pylori infection in dyspeptic patients in Yemen was (82.2%) [21]. Moreover, the seroprevalence of H.pylori antibodies was (9%) among Yemeni children [22]. Al - Makdad et al (2013) mentioned that the prevalence of H. pylori infection in hospital patients undergoing upper gastrointestinal endoscopy is very high (98.7%) [23]. Also Mayass (2013) found that the total prevalence of H.pylori antibodies in blood were (82.5 %), while the prevalence of H. pylori in stool according to H.pylori antigen test and H. pylori isolation were (18.5 %) [24]. Several modes of transmission of H. pylori have been described in the literature; these included direct contact between subjects, considered the most common mode, contaminated water sources and food, and less commonly iatrogenic transmission (during endoscopies and dental care) and zoonotic transmission [25, 26]. Environmental or animal reservoirs were investigated as sources of H.pylori infection. Food, animals, and water sources have been suggested as reservoirs outside the human gastrointestinal tract, and H.pylori or its DNA was detected in each source [27]. Food is a credible source of H.pylori infections [28, 29]. Several studies address the role of food in the transmission of H.pylori. Food products analysed are mainly milk, meat and vegetables. Among these, milk products are the most studied, probably because the infection is mainly acquired during childhood and consumed during this period [30]. The role of foods in the transmission of H.pylori is still unknown but there were several investigations which focused on the identification of this bacterium in various types of food samples. However, there is an increasing need for comprehensive studies emphasizing the significance of H.pylori as a food-borne pathogen [31]. Also numerous epidemiological studies have reported positive associations between untreated or faecally contaminated drinking water and incidence of H.pylori infection [32, 33]. Various findings support the hypothesis of H.pylori water-borne infection.

Chen et al. [34] and Yahaghi et al. [35] mentioned that individuals who consume raw vegetables are more likely to acquire H. pylori. The association of the infection with the consumption of raw vegetables is additional indirect evidence of the presence of H. pylori in water used for irrigation of these vegetables [30].

The role of foods in the transmission of H.pylori is still unknown, but there are numerous studies on its isolation from several types of food like milk, vegetables and Salad [35, 36, 37]. Favourable conditions for the growth of bacteria in different types of foods, including ready to eat foods, meat, salads and vegetables, would improve H.pylori existence [35, 37].

Molecular epidemiology studies had detected H.pylori DNA in different foodstuffs, water, and animals, suggesting reservoirs for H.pylori outside the human gastrointestinal tract [37, 38]. Milk, meat, and vegetables are potential sources of H.pylori infections [39, 40]. Milk products are the most studied, probably because the infection is mainly acquired during childhood and milk is consumed during this period [41, 42]. 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-faecal route [13].

Despite the absence of solid evidence of foods as a reservoir for H. pylori, different studies had isolated different strains and raised concerns about the contribution of nonhuman sources [42, 43, 44]. Suboptimal sanitation conditions are favoured for oral-faecal & oral-oral transmission of H.pylori in disabled individuals and orphanages [13]. 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 for humans [45]. Foods intrinsic factors, such as pH ranging (4.9 to 6.0) and aw (>0.97), could theoretically provide suitable 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 [8, 45, 46].

Due to the variety of risk factors present in developing countries, infection with multiple H.pylori genotypes is highly prevalent in the Middle East and North Africa (MENA) region. The incidence of H.pylori infection in Yemen is increasing; it is believed to be transmitted primarily by faecal-oral or oral – oral routes, with food and water as possible vehicles of infection; therefore, the objectives of this study were to monitor the occurrence of H.pylori in ready to eat foods (RTE) in Dhamar Governorate and to determine the relationship between the incidence of H.pylori in RTE foods with months during the period of study. Furthermore, studying the epidemiological data on H.pylori help in creating public health action that could stop transmission and, therefore, achieve the infection and aid the therapeutic timetable to eradicate the bacterium.

2. Materials and Methods

2.1. Study Design and Sampling

From January to June 2021, two hundred and sixty (280) ready-to-eat food samples (85 Chicken sandwich samples, 80 Red meat sandwich samples, and 115 salad samples) were collected from Restaurants and Street vendors in different places Dhamar Governorate. The samples were put in sterile cooled polyethene bags and kept in an icebox with a temperature of approximately 4°C during transport and store at laboratory [47].

2.2. Isolation of H.pylori

In the laboratory, the isolation of H. pylori was done under aseptic conditions as previously published [41, 48]. Briefly, samples were cut into small pieces using sterile blades to liberate adherent bacteria. 25 gm (as the optimal sample size) was soaked in 250 ml of normal saline from thigh and breast. 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 enriched in a microaerophilic atmosphere (GasPack; Oxoid, Basingstoke, England) at 37°C for 3 to 7 days. After that, modified Campy-blood 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.

2.2. Identification of H.pylori

According to a published standard scheme, the identification of H.pylori isolates was identified [41, 49]. 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, H2S production in (TSI), TSI with lead acetate paper, resistance to nalidixic acid, sensitivity to cephalothin, and hippurate

hydrolysis tes, Table 1. Isolates that met the typical results were considered H.pylori.

Table 1: Biochemical tests for the confirming of H. pylori.

Biochemical	Tests Results
Catalase	Positive
Oxidase	Positive
Urease	Positive
Indole	Negative
Growth in 1% Glycine	Negative
Growth in 3.5% NaCl	Negative
H2S Production in (TSI)	Negative
TSI with lead acetate paper	Positive
Nalidixic acid	Resistance
Cephalothin	Sensitive
Hippurate Hydrolysis	Negative

2.3. 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.

3. Results

3.1. Prevalence of H. pylori in RTE foods samples

From 280 ready to eat samples, Chicken sandwich samples, 85 (10.6%) showed a positive result for H.pylori, Red meat sandwich 80 (13.8 %) positive samples and 115 (14.8%) positive samples from Salad, Table 1. There is no significant difference between the isolation of H.pylori from different types of RTE foods and months.There is no significant difference between sample types in terms of contamination with H.pylori ($p = 0.71$) Based on this sample size, up to 14% of salad food samples are expected to be contaminated with H. pylori.

3.2. Prevalence of H. pylori in RTE foods according to the source of samples

Regarding the distribution of H.pylori among examined samples, the results showed a slightly higher prevalence of H.pylori in samples from Street vendors, Table 2. However, this increase was not significant statistically (Chi-Square = 0.39, $p = 0.83$).

4. Discussion

Various findings support the hypothesis of H.pylori food-borne infection. H.pylori has been identified in different types of food and in water sources (7,33). In the study at hand, two hundred and eighty 280 (85 Chicken sandwich samples, 80 Red meat sandwich samples and 115 salad samples) ready to eat food samples were collected among Dhamar Governorate, Yemen, during the period from January to June 2021. As far as we know, this is the first report of isolation of H.pylori from various types of RTE foods in Yemen. The overall prevalence of H.pylori was 37/280 (13.2%), Table 1, which is higher than results of Rahimi and Kheirabadi (2012) that reported the rate of contamination in milk was (0.67%) (50) and also higher than the results confirmed by Gilani et al. (2017) that mentioned the percent of pollution in meat samples was (5.0%) [51].

Our result was consistent with Atapoor et al. (2014) that found the total isolation rate of H.pylori from vegetables and salad samples were 44 out of 460 (9.56%) using the culture method, while the Polymerase Chain Reaction technique showed that 50 of 460 samples (10.86 %) were positive for H.pylori [36]. Al-mashhadany and Mayass (2017) found the prevalence of H.pylori among different types of food was 11.7% [41]. Yahaghi et al. (2014) found that 7 out of 50 (14.00 %) salad and 52 out of 380 (13.68%) vegetable samples harboured H.pylori [35].

In addition, our result was compatible with the study conducted by Talaei et al. [26] which found the frequency of H.pylori in ruminant raw milk samples were 28 out of 210 (13.33 %), and with Kianpour et al. [52] reported that the H. pylori ureC gene were detected in 24 (11.4%) of buffalo milk samples. In Table 1, we noticed that the highest rate of pollution with H.pylori was found in Salad 17/115(14.8%), then in red meat sandwich 11/80(13.8%), while the lowest rate of occurrence was found in Chicken sandwich 9/85(10.6%). The study conducted by Ghorbani et al. [53] reported that the prevalence of H.pylori in food items was 20.0%. They showed that vegetable sandwiches (45.0%), minced meat (32.0%), and meat sandwiches (20.0%) were the most commonly contaminated samples.

Table 2: Prevalence of H. pylori in Different Types of Ready to Eat Foods

RTE Foods	No. samples	+ve Samples No.%		-ve Samples		Chi-Square	Asymp. Sig.
Chicken sandwich	85	9	10.6	No.	%	0.68	0.71
Red meat sandwich	80	11	13.8	76	89.4		
Salad	115	17	14.8	69	86.2		
Total	**280**	**37**	**13.2**	**98**	**85.2**		

Table 3: Prevalence of H. pylori in RTE Foods according to the source of samples

RTE	Restaurants				Street vendors			
	No. of tests	Positive samples n (%)	Chi-Square	Asymp. Sig.	No. of tested	Positive samples n (%)	Chi-Square	Asymp. Sig.
Chicken sandwich	40	4 (10.0)	0.29	0.87	45	5 (11.1)	0.39	0.83
Red meat sandwich	40	5 (12.5)			40	6 (15.0)		
Salad	50	7 (14.0)			65	10 (15.4)		
Total	**130**	**16 (12.3)**			**150**	**21(14.0)**		
Chi-Square	**0.15**							
Asymp. Sig.	**0.70**							

Table 4: Relation between Months and Prevalence of H. pylori in RTE Foods

Month	Chicken sandwich No.	Positive %	Red meat sandwich No.	Positive %	Salads No.	Positive %	Total exam. Positive (%)		Chi-Square	Asymp. Sig.
January	14	1(7.1)	13	1(7.7)	19	2 (10.5)	46	4(8.7)	3.31	0.65
February	12	1((8.3)	15	2(13.3)	17	2(11.8)	44	5(11.4)		
March	14	1(7.1)	14	1(7.1)	19	2(10.5)	47	4(8.5)		
April	15	2(13.3)	13	3(23)	20	4(20)	48	9(18.6)		
May	16	2(12.5)	12	2(16.7)	19	4(21)	47	8(17.0)		
June	14	2(14.2)	13	2(15.3)	21	3(14.2)	48	7(14.6)		
Total	**85**	**9 (10.6)**	**80**	**11 (13.8)**	**115**	**17(14.8)**	**280**	**7(13.2)**		

Also prevalence level of H.pylori in food samples was higher than that of Rahimi and Kheirabadi [50] (0.67% in milk samples), Gilani et al. [51] (5% in meat samples), Atapoor et al. [36] (9.56% in vegetable), and Al-Mashhadany and Mayass [41] (11.74% in food samples). While it was lower than Ghorbani et al. [53] reported that the prevalence of H.pylori in food items was 20%. They showed that vegetable sandwiches (45%), minced meat (32%), and meat sandwiches (20%) were the most commonly contaminated samples. One possible reason for the high prevalence of bacteria in the vegetable sandwich was there not much time for good washing of vegetables used in the sandwich. In addition, vegetables are mainly grown in animal manure and irrigated using polluted water. The role of animal faeces as a source for transmission of H.pylori has been reported previously. The results of Table 2 show that the prevalence of H. pylori in RTE foods collected from restaurants was 16/130 (12.3%), the distribution of H. pylori in chicken sandwiches, red meat sandwiches, and salad were (10.0%), (12.5%), and (14.0%) respectively, while the prevalence of H.pylori in RET foods collected from street vendors was (14.0%), the distribution of H. pylori in Chicken sandwich, Red meat sandwich, and Salad were (11.1%), (15.0%), and (15.4%) respectively. Our result was compatible with the study

conducted by Talaei et al. [26] which found the frequency of H. pylori in ruminant raw milk samples were 28 out of 210 (13.33 %), and with Kianpour et al. [53] that reported the H. pylori ureC gene was detected in 24 (11.4%) of buffalo milk samples. Rahimi and Kheirabadi [51] reported that three of 447 milk samples (0.67%), including two sheep (2.2%) and one buffalo (1.6%) milk samples were found to be contaminated with H. pylori by using the cultural method, while H.pylori ureC gene was detected in 56 (12.5%) of milk samples, including 19 cow (14.1%), 11 sheep (12.2%), nine goat (8.7%), two camel (3.6%), and 15 buffalo (23.4%) milk samples. Many researches were inconsistent with our finding, Dore et al., (55) found that H.pylori was detected in 60.0% (38/63) of milk samples and 30% (6/20) of sheep tissue samples. Meng et al. (56) found that H.pylori was detected with multiplex polymerase chain reaction (PCR) in 36 % (4/11) of the raw chickens and 44 % (8/18) of the ready-to-eat raw tuna meat. In the study conducted by Mousavi et al. (37) observed that 19.8% of milk samples (103/520) and 19.2% of dairy products samples (77/400) were contaminated with H.pylori. The most frequently contaminated samples were ovine milk (35%) and traditional cheese (30%), and the prevalence of vacA, cagA, iceA and oipA factors were 75.0 %, 76.6%, 41.6% and 25.0%, respectively. El-Dairouty et al. (31) reported that the distribution of H.pylori in raw meat, raw poultry meat, and luncheon meat was (3.3 %), (5.0 %), and (5.0 %) respectively. Saeidi and Sheikhshahrokh [7] illustrated that the distribution of H.pylori in total samples of raw milk and meat was 24.02% (197/820) including 21.90% (92/420) raw milk and 26.25% (105/400) raw meat. According to our results and previous studies, H.pylori occurs in foods.

Thus, it is highly probable that H.pylori could contaminate foods and survive in these foods for some time, being transmitted to those who consume them. This supports the propounded oral-oral and faecal-oral modes of transmission. Methods for the direct culture of H.pylori haven't been fully developed. Part of the difficulty in detecting this pathogen is that changes in cell morphology, metabolism, an growth patterns occur when the organism is exposed to different environmental stimuli, including the condition of viable but non-culturable organisms.

This study exhibited the relationship between months and H.pylori incidence during research in Dhamar Governorate. Table 3 showed that the highest rate of isolation of H.pylori from different kinds of RTE foods was found in April (18.6 %), approximately close to this rate in May (17.0 %). But the isolation rate was seen decrease whenever moved away from these three months and in both directions. There is no significant difference between the isolation of H.pylori from red meat and months ($p > 0.05$).

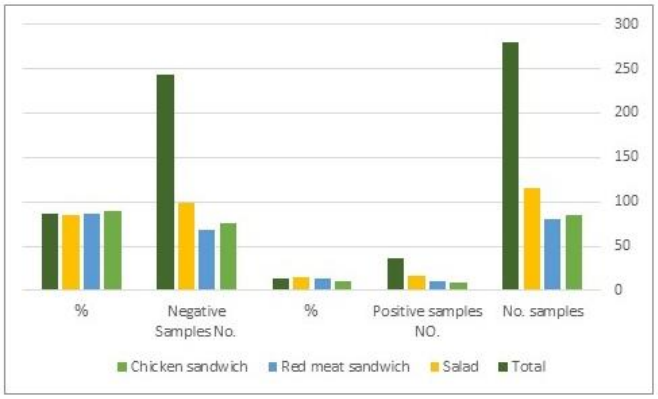

Fig. 1: Prevalence of H. pylori in different types of ready to eat foods.

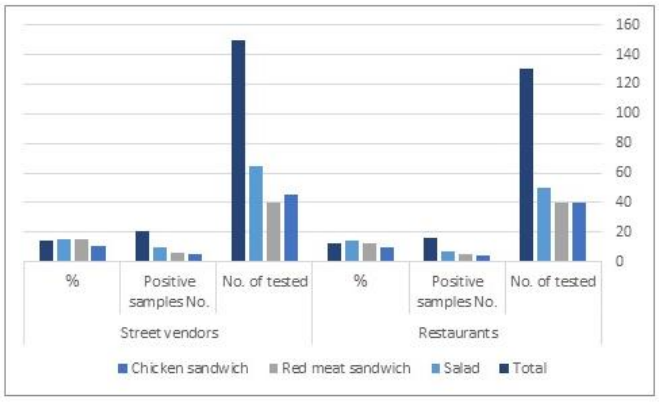

Fig. 2: Prevalence of H. pylori in RTE foods according to the source of samples.

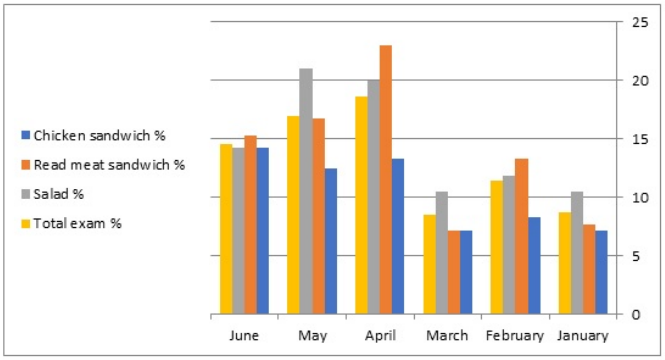

Fig. 3: Relation between months and Prevalence of H. pylori in RTE foods.

5. Conclusions

In this study, we concluded that the prevalence of H.pylori in RTE foods in Dhamar Governorate seems to be high (13.2%), this high percentage may be due to poor living conditions, family dietary, socioeconomic status, and sanitary habits , or another risk factors that can increase the occurrence of infection. Many incidences of H. pylori proposes that contaminated chicken sandwich, Red meat sandwich, and salad may be the sources of H. pylori and their pathogenic genotypes. Also in this study, Yemeni people exhibit that had a significant public health issue which should be addressed to stop the spread of the H. pylori infection. The dissemination of health awareness through media (audio, visual media, and newspapers) highlighting the transmission mode of these bacteria is highly recommended.

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