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Helicobacter pylori and its Interrelations with other Foodborne Pathogenic Bacteria in Egyptian Meat and some Meat Products

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ABSTRACT

Helicobacter pylori incidence was investigated in a total of 70 meat and meat product samples comprising raw meat (30), raw poultry meat (20) and luncheon meat (20) samples, randomly collected from different areas in Cairo and Giza markets. The interrelation between *Helicobacter pylori* and other foodborne pathogenic bacteria was studied in meat samples proved to contain *Helicobacter pylori*. Results revealed that one sample of each of raw meat (3.3%), raw poultry meat (5%) and luncheon meat (5%) was found to contain *Helicobacter pylori*, equally, using the two isolated media, (HPSPA) and (BHIB).

Positive Raw meat sample for *H. pylori* was found to yield total aerobic colony count (TACC) 5.47 log cfu/g, and contain coliform bacteria, *Bacillus cereus* and *Staphylococcus aureus*. Molds were not detected in the sample. Raw poultry meat sample positive for *Helicobacter pylori* was found to yield TACC 2.30 log cfu/g, and negative for molds, foodborne pathogenic Gram- negative and Gram- positive bacteria. The positive luncheon meat sample for *Helicobacter pylori* was found to yield TACC 5.27 log cfu/g, and free from molds, food borne pathogenic Gram- negative and Gram – positive bacteria.

Hence, there was 33.3% interrelation for the presence of *Helicobacter pylori* with other foodborne pathogens, coliform bacteria, *Bacillus cereus* and *Staphylococcus aureus* in meat and meat products. Also, results revealed that there was no difference between the two isolated media HPSPA, and BHIB, and the two identification methods, traditional biochemical method and rapid method (HiAssorted^{tm}, Biochemical kits**)** for *Helicobacter pylori* recovery.

Key words: Helicobacter pylori, interrelation, foodborne pathogenic bacteria, meat, poultry meat,luncheon meat.

Introduction

Helicobacter pylori is a major human pathogen causing chronic gastritis and has associated with several serious diseases of the gastrointestinal tract, including duodenal ulcer, gastric cancer in adults and children (Banerjee *et al.*, 1994 and Wong *et al.,* 2005). However, the origin and transmission of this bacterium has not been clearly explained. 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 (Atapoor *et al*., 2014; Yahaghi *et al*., 2014 and Hemmatinezhad *et al*., 2016) and considered it to be a food borne pathogen (Meng and Doyle, 1998).

Much interest has been noticed for studying *H. pylori* incidence in the Egyptian foods and its ecological interactions with other foodborne illness bacteria due to the increase of associated illness in Egyptian cases (Metwally *et al*., 2001). The worldwide distribution of *H. pylori* and prevalence is about 70-90% in North Africa. *Helicobacter pylori* has been isolated from drinking water (Queralt *et al.,* 2005), raw vegetables (Gomes and de Martinis, 2004), foods of animal origin (Dore *et al*., (2001) and cow milk (Fujimura *et al*., 2002). Though, these findings suggested that these foods may act as vehicles of transmission. The ability of *H. pylori* to survive in common foods supports the hypothesis that primary contamination of a food product (animal reservoir) or secondary contamination due to inappropriate handling (human reservoir) can be a vehicle for *H. pylori* transmission (Quaglia *et al.,* 2007).

Meng *et al*. (2008) reported that by using novel multiplex PCR system for *H. pylori* detection in eleven raw chicken samples at 4 C (whole chicken with skin) obtained from a grocery in the Chicago area, 36% of the fresh raw chickens were *H. pylori* positive. They also found highly probable that *H. pylori* could contaminate foods and survive for some time, and being transmitted to those who consume it. Nouraie *et al.* (2009) found that food handled inappropriately, as a risk factor, is a consequence and associated with socioeconomic development, which in epidemiology studies usually acts as a confounding factor. The improvement of general hygienic conditions decreases the prevalence of the infection.

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In Egypt, Saad and El Prince (2004) studied the prevalence of *H. pylori* and other *Helicobacter* species in raw milk and some milk products in Assiut governorate. They could isolate *Helicobacter* species from raw marketable milk, Kariesh cheese and cooking butter samples. Poms and Tatini (2001) tested the survival of *H. pylori* in semi-processed ready-to-eat foods and one raw chicken and observed the survival of *H. pylori* at 4ºC under aerobic conditions. Also, they found that *H. pylori* was recovered from spiked pasteurized milk and tofu samples up to 5 days later and from spiked leaf lettuce and raw chicken up to 2 days later. Among the main reasons for the presence of *H. pylori* in meat and meat products are two factors namely primary contamination of meat and cross contamination.

Hence, the main objective in this study was to trace the incidence and frequency distribution of *H. pylori* in raw meat, raw poultry meat and luncheon meat samples, collected from Cairo and Giza markets, using different media and rapid identification kits. Also, investigation of the microbe ecological interrelations with other foodborne pathogenic bacteria in these foods was a target.

Materials and Methods

Samples collection:

A total number of 70 samples including raw meat (30 samples), raw poultry meat (20 samples) and luncheon meat (20 samples) were randomly collected from markets of different locations in Cairo and Giza governorates, Egypt and delivered directly, in sterile bags/ ice box, to the laboratory for microbiological analysis**.**

Sample preparation:

Twenty five grams of each of meat, poultry or luncheon samples were homogenized with 225 ml sterilized buffer peptone water in sterile blender cups individually.

Isolation and identification of Helicobacter pylori:

I. Isolation of Helicobacter pylori:

a. Isolation using Helicobacter pylori special peptone broth & agar (HPSPB, A) media:

According to the technique adapted by Stevenson *et al*., (2000), Diluted samples were aseptically inoculated into sterile test tubes containing the selective enrichment broth (HPSPB) supplemented with 5% sterile horse serum and *Helicobacter pylori* selective supplement (SR0147E) (5mg/l vancomycin, 2.5mg/l trimethoprim, 2.5mg/lcefsulodine and 2.5mg/lamphotericine B). The inoculated tubes were incubated at 37°C for 48h in an atmosphere of 6% O_2, 10% CO_2 and 84% N2, using an anaerobic jar and gas generating kits (Oxoid BR 56). A loop full of the incubated enrichment broth of each sample was streaked on *Helicobacter pylori* special peptone agar (HPSPA) supplemented with 5% sterile horse serum, *Helicobacter pylori* selective supplement (SR0147E). Streaked plates were incubated at 37°C for 3-7 days under anaerobic condition. All the cultured plates were inspected after 3, 5 and 7 days. Suspected colonies grew slowly and appeared small not exceeding 2 mm in diameter, circular, convex and translucent. Such colonies were picked up by a platinum loop and retreated onto selective media and anaerobically incubated at 37°C.

b. Isolation using brain heart infusion (BHI) media:

Similarly and simultaneously, diluted samples were analyzed using the media of Brain Heart Infusion Broth (BHIB) and Agar (BHIA) with supplements (Oxoid SR0147E), pursuant to the modified procedure described by Quaglia *et al.,* (2007). Plates were incubated for 5-10 days at 37ºC under microaerophilic condition. Circular, convex and translucent colonies were picked up, retreated onto selective media and anaerobically incubated at 37°C to be identified.

II. Identification of *Helicobacter pylori:*

Identification of *Helicobacter pylori* isolates was carried out using both the traditionally biochemical tests and biochemical identification test kits ("Hibio-ID") using HiAssorted™ Biochemical test kits (HiMEDIA, KB002), HiMEDIA laboratories Pvt. Limited, A-406 Bhaveshwar Plaza, Mumbai-400 086, India. The obtained isolates were identified according to their morphological culture, physiological and biochemical characteristics as described by Zenner (1999).

a. Colony morphology:

Colonies were grown at 37C and appear circular, convex, translucent and not exceed 2mm in diameter (Naclmmkin and Skirrow, 1998).

b. Biochemical reactions:

The bacterial culture was biochemically identified according to catalase, oxidase, Urease, Hipurate hydrolysis test, nitrate reduction tests and presumptive growth at 42°C (Finegold and Martin, 1982; Baron et al., 1994; Collee and Miles, 1996).

c. Biochemical identification kits of H. pylori:

Also, biochemical identification test kits ("Hibio-ID") using HiAssorted^{TM} Biochemical test kits (HiMEDIA, KB002), HiMEDIA laboratories Pvt. Limited, A-406 Bhaveshwar Plaza, Mumbai-400 086, India, was followed as a newly rapid method for identification of the isolates.

Interrelations between the incidence of H. Pylori and other food borne microorganisms:

The incidence of *Helicobacter pylori* in the previously different kind of meat and meat products was studied in relation to the presence of other bacteriological criteria such as: total aerobic colony count (TACC), mold and yeast count, Gram- positive bacteria comprising *Staphylococcus aureus*, *Bacillus cereus* and *Listeria monocytogens* and Gram- negative bacteria including coliform bacteria, *Yersinia enterocolitica, E. coli O157: H7* and *Salmonella* spp.

Total aerobic colony count (TACC):

Total aerobic colony count (TACC) was carried out due to the conventional method (FDA., 2002) using plate count agar (Oxoid).

Molds and yeasts count:

Enumeration of mold and yeast counts were carried out in the samples using the media of acidified potato dextrose agar (Mu96, Himedia, Mumbai). The method recommended by FDA (2002) was followed up.

Detection of Listeria monocytogens:

Tryptose soy broth (Fluka, Switzerland) supplemented with 0.5% yeast extract and listeria selective enrichment supplements (CoodeSR140) was used according to (Lovett *et al.,* 1987). Incubation of the flasks was done at 30 ºC for 7 day. Every day a plate of Listeria selective agar base (Oxoid CM856) with supplements were streaked from each of an enrichment flask and incubated at 35 ºC for 48h as reported by Curtis *et al.* (1989). Suspected colonies were further morphologically and biochemically tested as recommended by FDA (2002).

Enumeration of Staphylococcus aureus:

Enumeration of *S. aureus* in the samples was carried out using the Baird Parker agar medium (Baird-Parker, 1962) supplemented with egg yolk and potassium telurite solution as recommended by APHA (1976) and FDA (2002).

Enumeration of Bacillus cereus:

Bacillus cereus was determined by the surface plating technique onto *Bacillus cereus* selective agar (Oxoid) supplemented with egg yolk and polymyxin solution (SR99, Oxoid) (Holbrook and Anderson, 1980). Methods and identification were carried out according to FDA (2002).

Determination of coliform bacteria:

Coliform group was determined using solid medium method onto plates of violet red bile agar (VRBA) according to the method reported by FDA (2002).

Detection of Escherichia coli O157: H7:

Escherichia coli 0157:H7 was detected by using Sorbitol MacConkey agar medium (SMAC, Oxoid, England). Biochemical-serological identification was applied according to FDA (2002).

Isolation and identification of Salmonella:

Pre-enrichment, selective enrichment and selective plating on *Salmonella & Shigella* agar (SS agar) (Oxoid) plates were done. Lactose negative suspected Salmonella *or Shigella spp*. was biochemically and serologically identified according to the methods recommended by FDA (2002) and APHA (1976).

Detection of Yersinia enterocolitica:

Detection of *Y. enterocolitica* in the samples was carried out by using peptone sorbitol bile broth (PSBB,Oxoid code CM653) supplemented with Yersinia selective supplement (SR 109). After incubation, further specific biochemical tests were carried out according to the method and the media recommended by FDA (2002.

Statistical Analysis:

Statistical analyses were performed using the GLM procedure with SAS (2004) software. Duncan's multiple comparison procedure was used to compare the means. A probability to $P \leq 0.5$ was used establish the statistical significance.

Results and Discussion

Incidence and frequency distribution of *H. pylori* in raw meat, raw poultry meat and luncheon meat samples

A total number of 70 samples including 30 raw meat samples, 20 raw poultry meat samples and 20 luncheon meat samples (as one of popular and common meat products), collected from Cairo and Giza markets were investigated for the incidence of *Helicobacter pylori*.

Results in Table (1) revealed that *H. pylori* were isolated from raw meat samples in percentage of 3.33 %, equally, using both of the two selective media. Also, *H. pylori* were isolated from raw poultry meat and luncheon meat samples in percentage of 5.0 % each, equally, using both of the two selective media HPSPA and BHIA.

The same frequency of *H. pylori* given by the two HPSPA and BHIA selective media in raw meat, raw poultry meat and luncheon meat samples were given by the conventional and rapid biochemical (Hibio-ID", HiAssorted^{tm} Biochemical test kits KB002) identification methods as shown in Table (2). The obtained results are generally not contradict, but show much lower percentages comparing with the results reported by Meng *et al*., (2008) who used more advanced novel multiplex PCR system to test eleven raw chicken samples (whole chicken with skin) and found 36.0% (4/11) of the samples were *H. pylori* positive. Also, Poms and Tatini (2001) emphasized the survival *of H. pylori* in chicken, since they found that *H. pylori* was recovered from spiked raw chicken and survived at 4^{o}C under aerobic conditions up to 2 days later.

Thus, it is highly probable that *H. pylori* could contaminate meat and meat products, survive in or on these foods for some time, and transmit to the consumer. This supports the propounded fecal-oral modes of transmission as it is unlikely postulated that *H. pylori* resides in living animals and chicken. Also it is proposed that *H. pylori* isolated from local samples of raw meat and raw chicken meat and luncheon meat obtained from butchers or grocery were come from slaughtered houses, shops or factories when these foods were slaughtered and/or processed. Humans are the definitive natural reservoirs of *H. pylori*, thus, it is likely to consider workers, chefs, water, tools and equipment that comes in close contact with meat and meat products may be the original source of the *H. pylori* contamination (Meng *et al.*, 2008 and Wong *et al.*, 2005).

The ability of *H. pylori* to survive in common foods supports the hypothesis that primary contamination of a food product (animal reservoir) or secondary contamination due to inappropriate handling (human reservoir) can be a vehicle for *H. pylori* transmission (Quaglia *et al.,* 2007). Moreover, studies on the relation between the form presence and virulence reported that, *H. pylori* in adverse environments can transform into a viable but non cultural state, a coccoid form (Bode *et al*., 1993 and Chan *et al.*, 1994). It has been speculated that the coccoid form of *H. pylori* plays a role in the survival of the bacterium outside the human host, which may contribute to the transmission of *H. pylori* from an environmental source. Wang *et al.* (1997) reported that the coccoid form

possesses the same enzymes and proteins that have been defined as virulence factors in spiral form**;** therefore, coccoid forms of *H. pylori* may be important in food-borne transmission of the bacteria.

Table 1: Incidence of *H. pylori* in raw meat, poultry meat and luncheon meat samples collected from Cairo and Giza markets

Types of samples	Number of analyzed samples	Positive samples			
		HPSPA		BHIA	
		Number	%	Number	%
Raw meat	30	1	3.33	1	3.33
Poultry meat	20	1	5	1	5
Luncheon meat	20	1	5	1	5
Total samples	70	3	4.28	3	4.28

Table 2: HiAssorted[tm] rapid biochemical kits and conventional methods for *H. pylori* identification

Source of isolates	Number (%) of the isolates identified by traditionally biochemical test		Number (%) of the isolates identified by HiAssortedtm biochemical kits	
Raw meat samples	1/30	3.33	1/30	3.33
Poultry meat samples	1/20	5	1/20	5
Luncheon meat samples	1/20	5	1/20	5
Total samples	3/70	4.28	3/70	4.28

Interrelations between the incidence of *H. Pylori* and other food borne microorganisms in raw meat, raw poultry meat and luncheon meat samples

Results in Table (3), illustrate the relationship between *H. pylori* and other food borne microorganisms in positive samples of meat and meat products.

In regard to raw meat samples, only one sample out of thirty (1/30) representing 3.3% was positive for *H. pylori*, and yielded count of 5.47 log cfu/g of TACC, whereas mold and yeast were not detected. Moreover, from the pathogenic foodborne Gram–negative bacteria, coliform bacteria were only found and yielded 4.47 log cfu/g. Meanwhile, from the foodborne pathogenic Gram – positive bacteria, *B. cereus* and *S. aureus* were isolated and yielded counts of 2.69 log cfu/g and 3.0 log cfu/g, respectively.

For raw poultry, The only one positive sample for *H. pylori* was found to yield 2.30 log cfu/g of TACC, but was free from mold and yeast, Gram-negative and Gram – positive foodborne pathogenic bacteria.

Concerning to luncheon meat, twentieth sample (5%) which was positive for *H. pylori* yielded 5.27 log cfu/g of TACC, whilst it was negative for either Gram – positive, Gram – negative foodborne pathogenic bacteria or molds and yeasts.

Though, the obtained results reveal that the presence of *Helicobacter pylori* was interrelated with other foodborne pathogens, coliform bacteria, *Bacillus cereus* and *Staphylococcus aureus* in 33.3% of investigated meat and meat products. Precisely, the coliform bacteria*, S. aureus and B. cereus* were of the most frequent only in the positive raw meat sample for *H. pylori* achieving 4, 47, 2.69 and 3 log cfu/gm, respectively. These results declared that in spite of the presence of *H. pylori* in poultry meat and luncheon meat samples they were in conform to the Egyptian Standard (ES1008-2005), they were free from other pathogenic bacteria. Whilst, the raw meat sample did not meet the Egyptian Standard (ES1008-2005). This may urge and direct the Egyptian standards of meat and meat products to involve *H. pylori* as a bacterial criterion for human fitness and standard conformity.

According to the obtained results, the total aerobic colony count (TACC) in all of the three positive samples for *H. pylori* were: 5.47 log cfu/gm in meat sample, 2.30 log cfu/gm in poultry meat sample and 5.27 log cfu/gm in luncheon meat sample. This was in concomitant with the findings obtained by Fasanmi and Sansi (2008) and El Gamal *et al*., (2012) who reported that the microbial contamination of meat and meat products must not exceed levels which could adversely affect human consumption. For food-borne pathogens, the results were not in concomitant with those obtained by Rashed *et al*., (2014) in respect of the presence of *Listeria monocytogens* in meat and meat products in Egypt. On the other hand, the obtained results were in agreement with Fasanmi *et al.,* (2010) who found that total of 27 strains of bacteria were isolated from meat samples, as *Escherichia coli*. It has being the most frequent bacteria and accounted for 13% of the total bacterial count, followed by *Enterococcus faecium* and *Bacillus cereus* which were accounted for 10% and 9% of the bacterial count, respectively. *Salmonella* species was accounted for approximately 2% of the total bacterial count in the study. Also, Fasanmi and Sansi, (2008) reported coliform bacteria as the highest occurring bacteria and *E. coli* was the major meat contaminant. Moreover, Olawale *et al*. (2005), reported nine bacterial genera including

Staphylococcus aureus, Aerobacter aerogenes, Enterococcus faecalis and E. coli, in the presence of *H. pylori,* isolated from food samples and stalls in public canteens.

Table 3: Relationship between the incidence of *H. pylori* and other food borne pathogens in raw meat, raw poultry meat and luncheon meat samples collected from Cairo and Giza markets.

Samples	No.of positive samples / Total samples	Gram-positive bacteria			Gram-negative bacteria				TACC Log cfu/gm	Mold & yeast Log cfu/gm
		L. monocytogen Log cfu/gm	*B. cereus* Log cfu/gm	*S. aureus* Log/gm	*Coliform* Log cfu/gm	*E.coli 0157:H7* Log cfu/gm	*Salmonella spp*	*Y. enterocolitic*		
Luncheon meat	1/20	0.0a	0.0b	0.0b	0.0b	0.0a	ND	ND	5.27a ± 0.11	0.0a
Raw meat	1/30	0.0a	3.00a ± 0.10	2.69a ± 0.15	4.47a ± 0.06	0.0a	ND	ND	5.47a ± 0.1	0.0a
Raw poultry meat	1/20	0.0a	0.0b	0.0b	0.0b	0.0a	ND	ND	2.30a± 0.06	0.0a
No. of total samples	3/70	0/3	1/3	1/3	1/3	0/3	0/3	0/3	3/3	0/3
(%) of positive samples	4.28	0	33.3	33.3	33.3	0	0	0	100	0

ND (not detected), + (positive).
Means with the same letters are not significantly different (p≤ 0.05). Values are means ± standard error.

This poses a serious public health issue for consuming raw meats and meat products, as *H. pylori* was an indicator of the bad conditions of hygienic practices at the various meat stalls in Cairo and Giza area. The present study expect that the distribution of raw meats and meat products, might have been associated with other diseases resulted from the prevalence of *H. pylori*, and much concern must be paid.

Conclusion

In Egypt, raw meat, poultry meat and luncheon meat products were found to harbor *H. pylori.* Therefore, consumption of these food products by people may be a source of *H. pylori* infection. So, emphasis on good hygiene could be a measure to reduce the load of this microbe in these products and subsequently to human.

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