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CAMPYLOBACTER CONTAMINATION IN RETAILED CHICKEN CARCASES FROM MANSOURA-EGYPT, AND ITS RELATION TO PUBLIC HEALTH

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ABSTRACT **The prevalence of Campylobacter spp. was investigated in 110 whole carcase samples of fresh retailed chickens (50), frozen chickens (25), stir-roasted chickens (15) and home-cooked chickens (20), purchased from Mansoura retial shops, supermarkets and restaurants. The incidences of Campylobacter contamination were 78%, 56%, 13.3% and 0% in fresh, frozen, roasted and home-cooked chickens, respectively. Campylobacter spp. were identified on the basis of colonial morphology, microscopic examination and biochemical tests. Campylobacter jejuni was more frequent than C. coli and C. laridis. 66%, 48% and 13.3% of fresh, frozen and stir-roasted chickens, respectively were positive for C. jejuni, while 16%, 12% and 6.7% of the examined chicken samples, respectively were positive for C. coli, and only 4% for each of fresh and frozen chickens were positive for C. laridis. Of the 133 identified isolates of Campylobacter, 100 (75.19%) were proved as C. jejuni, 28 (21.05%) proved as C. coli and only 5(3.76%) identified as C. laridis. The results indicated that fresh and frozen retailed chickens and, to lesser extent, roasted chickens are contaminated with enteropathogenic Campylobacter spp. and that could representing a potential health hazard for humans. The public health signficance of such pathogen as well as the suggestive recommended measures to control it had been discussed.**

INTRODUCTION

During the last decade, Campylobacter spp. have been recognized as one of the most common cause of acute diarrhoea or enterocolitis and the rate of its notification has continued to rise since 1992 (**Nielsen et al., 1997; and Lee et al., 1998**).

Campylobacters are motile, Gram–negative, slender curved or spiral rods, appearing vibroid . and are microaerophilic, growing best at gaseous atmosphere of 5-10% oxygen, 10% carbon dioxide and 85% nitrogen approximately (**ICMSF, 1996; and Harrigan, 1998**). The most important

pathogenic strains belong to the group of thermotolerant Campylobacters, are C. jejuni, C. coli and to lesser extent C. laridis and these species are isolated most often from human **(Griffiths and Park, 1990; and Shih, 2000)**.

The principal environmental reservoir of pathogenic Campylobacter is the intestinal tract of wild and domestic birds, therefore poultry is considered as the largest potential source of Campylobacter for human and the high optimum growth temperature of C. jejuni and C. coli could be an adaptation to the higher body temperature of birds **(Adams and Moss , 1995)**.

Poultry is differ from other food animals in that the skin of poultry is not normally removed during processing and the normal processing procedure allow cross contamination of carcases skin with Campylobacters from the intestine, feathers, feets, as well as equipment surfaces, worker hands and knives **(Izat et al., 1988; and Kotula and Pandya, 1995)**. So, Campylobacter spp. were detected at high frequencies (up to 100%) in poultry meat including fresh processed, chilled, frozen and even cooked carcases. A high count (over 10^8 Campylobacter cells) could be estimated on the surface of one poultry carcase **(Humphrey et al., 2001)**.

The consumption of raw or under-cooked poultry has been implicated in high numbers of outbreaks of acute Campylobacter enterocolitis in human worldwide in both industrialized and developing countries, especially in children, the eldery and immunosuppressed patients **(Blaser and Reller, 1981, Florin and Antillon, 1992; Baffone et al., 1995; Nielsen et al., 1997; and Quinones-Ramirez et al. 2000)**. In England and USA, Campylobacter enterocolitis is more frequent than Salmonella **(Lacey, 1993; Meer and Misner, 1998; and Pearson et al., 2000)** and it is causing over 50000 confirmed cases of infection in England and Wales each year **(Humphrey, 2001)**.

In Egypt, it is common that the consumers purchase freshly-processed chicken carcases from poultry retail shops and either consume it directly after cooking or kept them in domestic freezers (-10 : -20 °C) for several days to be ready at need. Also, in some instances, ready-to-serve, stir-roasted and grill-fried chickens are preferred by some consumers.

Therefore, this study was undertaken to determine the incidence of Campylobacter spp. in fresh and frozen chicken carcases and to estimate the existence and survivability of such organisms in ready-to-eat stir- roasted and home-cooked chickens.

MATERIALS AND METHODS

Sampling: One hundred and ten samples, including 50 freshly-processed , 25 deep-frozen (-15 : -20 °C), 15 stir- roasted and 20 home-cooked whole broiler chicken carcases (7-8 weeks old), were

collected over 3 months from poultry retail shops, supermarkets and restaurants in Mansoura City, Egypt. Collected samples were placed individually in sterile plastic bags and transferred to the laboratory within 1 hour of purchase or post-cooking. At the laboratory, all samples were held at 5 °C and subjected to the bacteriological examination within 2 hours. For frozen chickens, they were allowed to thaw in their packages at refrigerator temperature (5-7 °C) prior to examination.

Procedure for isolation of Campylobacter spp.

Current conventional methods for detection of Campylobacters in foods involve selective enrichment followed by plating onto selective media and biochemical confirmation **(Baylis et al., 2000)**.

(i) Preparation of sample. Since the microbial flora of poultry is confirmed largely to the skin surface, surface-sampling techniques are the most appropriate useful methods in the examination of fresh and frozen chickens after thawing **(Harrigan, 1998)**, and although chicken carcase wash (obtained by massaging and shaking the whole carcase with a suitable enrichment broth for 2 minutes in a sterile plastic bag) is a common method for sampling for Campylobacter recovery from chickens **(Stern et al., 1995 and Baylis et al., 2000)**, it is not applied here because of most of the bacterial contamination is found deep in skin tissues in the feather follicles (which allow microaerophilic condition for Campylobacters), and instead of swabbing or chicken-wash methods, samples are excised from the skin aseptically at different sites of chicken carcase **(Kiss, 1984)**.

(ii) Enrichment. Bolton selective enrichment broth (BB) (Oxoid, CM 983) with its supplement (SR 183) and laked horse blood (SR 48) was prepared according to the manufacturer instructions and used for enrichment of chicken samples. This selective broth (BB) was reported as the superior to any other enrichment broth used for isolation of Campylobacters from naturally-contaminated food **(Baylis et al., 2000)**.

25 grams composite of chicken skin (15 g) and meat (10 g) were excised from both breast and thigh regions of each carcase and added to 225 ml of BB, then homogenized for 1 minute in a stomacher (Colworth Stomacher, Seward Ltd., London, UK.). The homogenates were transferred to a screw-copped bottles leaving very little head space above the liquid (1.5–2 cm) to ensure microaerobic conditions. Then the bottles were incubated aerobically at 37 °C for 4 hours followed by 42 °C for 48 hours **(Baylis et al., 2000)**.

(iii) Media. Campylobacter blood-free selective medium (modified CCDA-Preston) was obtained from Oxoid consisting of Campylobacter blood-free selective agar base (CM739) and CCDA selective supplement (SR155). This medium is recommended for isolation of Campylobacter jejuni, C. coli and C.

laridis at 37°C, and it is specified by the U.K. Ministry of Agriculture, Fisheries and Food **(MAFF, 1993)** for isolation of Campylobacter from food. The medium was prepared according to the manufacturer instructions. Then a loopful from each enrichment was subcultured onto the medium and incubated at 37 °C for 24-48 hours under microaerophilic condition achieved by using Campylobacter Gas Generating kits (Oxoid, BR 56) in conjunction with their catalyst containing anaerobic jars.

(iv)Identification of Campylobacter spp. The plates were then examined for typical Campylobater colonies and the criteria for presumptive identification were based on the colonial appearance. C. jeuni and C. laridis produce grey, moist, glossy flat spreading colonies with or without a metallic sheen, while C. coli tend to be creamy-grey in color moist with slightly raised shiny surface. **(Roberts et al., 1995; and Oxoid, 1998)**. The 3 species are positive for oxidase and catalase and can grow at 42°C not at 25 °C. Microscopic examination revealed Gram-negative, curved to spiral or S-shaped rods **(Hunt, 1992)**. The isolates were confirmed to the species level by the following traditional biochemical assays: catalase, oxidase, growth in 1% glycine, H_2S production, nitrate reduction, hippurate hydrolysis, antibiotic sensitivity to nalidixic acid and sensitivity to cephalothin **(Penner, 1988; Hodge et al., 1990; Hunt, 1992; and Roberts et al., 1995)**.

RESULTS AND DISCUSSION

Campylobacter spp. are considered a commensal organisms in chickens, and poultry may be the major reservoir of the human infection in developed countries **(Zhu et al., 1999)**.

Incidence of Campylobacter spp. in examined chickens.

(i)Freshly processed ready-to-sale chicken carcases: Thirty nine (78%) out of the 50 fresh carcases examined , were positive for Campylobecter spp. (Table 1). This findings agree with those recorded in chicken carcases from different countries including ; UK **(Corry and Atabay, 2001)**, USA **(Adams and Moss, 1995)**, China **(Shih, 2000)**, and Japan **(Tokumaru et al., 1991)**, while lower percentages of Campylobacter recovery (25-40%) were determined in chicken carcases elsewhere in the world by **Nouman et al., (1986)** in Egypt, **Dias et al., (1990)** in Brazil, **Castillo-Ayala et al., (1993)** in Mexico, **Madden et al., (1998)** in Northern Ireland, **Atanassova et al., (1998)** in Germany and **Uyttendaele et al., (1999)** in Belgium. On the contrary, higher percentages of recovery (95-100 %) were reported in poultry carcases from USA **(Stern and Line, 1992 ; and Eyigor et al., 1999)**, Turkey **(Yildiz and Diker, 1992)** and Taiwan **(Lee et al., 1994)**.

The comparatively high levels of contamination of fresh chicken samples in retail markets were due to poor sanitation conditions , and improper handling during chicken processing that considerably increased the number of the thermophilic Campylobacters on the skin surface and this increased the chances of further cross-contamination of carcases **(Shih, 2000)**.

Table (1): Incidence of Campylobacter spp. in examined chicken carcases.

Chicken carcase	No. of samples	Campylobacter positive samples	
		No.	%
Fresh	50	39	78
Deep-frozen	25	14	56
Stir-roasted	15	2	13.3
Home-cooked	20	0	0
Total	110	55	50

Table (2): Frequency of Campylobacter spp. distribution in the examined samples of chicken carcases.

Chicken carcase	No. of samples	C. jejuni		C. coli		C. laridis	
		No.	%	No.	%	No.	%
Fresh	50	33	66	8	16	2	4
Deep-frozen	25	12	48	3	12	1	4
Stir-roasted	15	2	13.3	1	6.7	0	0
Home-cooked	20	0	0	0	0	0	0
Total	110	47	42.7	12	10.9	3	2.7

Table (3): Number and percentages of identified Campylobacter isolates among the examined chicken carcases.

Chicken carcase	No. of identified isolates	C.jejuni		C.coli		C.laridis	
		No.	%	No.	%	No.	%
Fresh	88	67	76.1	17	19.3	4	4.5
Deep-frozen	39	28	71.8	10	25.6	1	2.6
Stir-roasted	6	5	83.3	1	16.7	0	0
Home-cooked	0	0	0	0	0	0	0
Total	133	100	75.19	28	21.05	5	3.76

(ii) Frozen chicken carcases . Although freezing has a harmful effect on Campylobacters in meats **(Kraft, 1992)**, it could not eliminate these organisms from poultry carcases but it would only reduce their population. **Beuchat (1987)** reported that C. jejuni could survive in chicken meat stored at -18°C for several months.

It is known that many domestic freezers operate at -20°C or slightly lower temperatures, with fluctuation in the range between 1 and 6°C **(Abu Ruwaida et al., 1996)**, and these temperatures will support the survival of Campylobacters when present on the chicken skin **(Lee et al., 1998)**. It is likely that the chicken skin is providing an appropriate microenvironment to protect C. jejuni possibley in the undulations, folds. and feather follicles where there is protein supply, and possibly also fatty acids and oils which inhibit the formation of ice crystals **(Lee et al., 1998)**. Fourteen samples (56%) out of the 25 frozen chicken carcases examined were positive for Campylobacter spp. (Table 1). A similar situation (60%) was reported in frozen chickens by **Svedhem et al., (1981)**. Other publications estimated lower incidence of Campylobacters in frozen chickens **(Norberg, 1981; Hood et al., 1988; and Kraft, 1992)**. Conversely, in a study conducted in Germany by **Loewenherz-Luning et al., (1996)** they found that up to 100% of frozen poultry were positive for Campylobacter jejuni. Therefore, Campylobacters found in contaminated poultry are well protected even though the food has been frozen.

(iii) Stir-roasted and home-cooked chickens . Although many of the reported isolation frequencies of Campylobacter from uncooked chicken are very high (up to 100%) , the frequency of recovery from cooked chicken is expected to be lower , as the bacteria are easily destroyed by conventional cooking methods , since the organism was not detected on fully cooked (internal temperature 74°C) poultry meat **(Acuff et al., 1986)**.

Two samples (13.3%) out of the 15 examined stir-roasted chickens were positive for Campylobacter and none of the 20 home-cooked examined carcases were positive for such organism (Table 1).

In another related study , high incidence of Campylobacter (27%) were detected in roasted chicken in Mexico **(Quinones-Ramirez et al., 2000)**, whereas **Asif and Bari (1992)** reported that 3.64% of cooked chickens were positive for Campylobacter.

Svedhem et al., (1981) suggested heating of food to at least 60°C for 15 minutes to destroy the organism, although **Gill and Harris (1984)** recommended minimal cooking of poultry at 87.8°C for 20 minutes to eliminate C. jejuni .

The positive results of stir-roasted chickens were attributed to cross contaminaton between raw and roasted chickens, in the kitchen, poor food hygiene or unproperly cooking.

Frequency of Campylobacter spp. distribution.

Campylobacter jejuni was detected in 33 (66%) , 12 (48%) and 2 (13.3%) samples of the examined fresh, frozen, and stir-roasted chicken carcases , respectively, while 8 (16%), 3 (12%) and 1 (6.7%) samples of such carcases, respectively were positive for Campylobacter coli. Although low incidence (4%) of C. laridis was reported in both fresh and frozen chicken carcases (Table 2). The incidence of C. jejuni in fresh chicken carcases in this study (66%) was close to that reported in USA (69.4%) by **Willis and Murray (1997).** Although lower incidences of C. jejuni (40-50%) in retailed chicken carcases were published by **Haba et al., (1985), Atanassova and Ring (1999)** and **Ono and Yamamoto (1999)** . On the other hand, much higher incidence of C. jejuni (90-100%) was reported in fresh chicken carcases in UK **(Atabay and Corry, 1997; and Pearson et al., 2000).** Campylobacter jejuni was detected at a lower incidence in frozen chickens than in fresh ones. In sweeden , frozen chicken from retail stores had an incidence of 22% for C. jejuni **(Norberg, 1981).**

Identification of Campylobacter isolates.

Out of the 133 biochemically identified Campylobacter strains isolated from the different chicken samples, 100 (75.19%) were proved as C. jejuni and 28 (21.05%) identified as C. coli and only 5 (3.76%) were C.laridis (Table 3). This result confirmend that C. jejuni was the most prominent species isolated among Campylobacters from examined retailed chicken carcases, followed by C. coli, whereas C. laridis constituted the lowest incidence among the isolates. These findings substantiate what had been reported in chicken carcases throughout the world by **Yildiz and Diker (1992)** in Turkey, **Manzano et al. (1995)** in Italy, **Osano and Arimi (1999)** in Kenya, **Eyigor et al., (1999)** in USA, **Shih (2000)** in China and **Wedderkopp et al., (2000)** in Denamrk. On the contrary, **Fernandez and Pison (1996)** in their investigations on frozen commerical chicken livers in Chile found that C. coli was isolated more frequently (78.6%) than C. jejuni (21.4%). However, **Madden et al., (1998)** in Northern Ireland could isolate both C. jejuni and C. coli in approximately equal numbers. Also, **Castillo-Ayala et al. (1993)** found that 50% of Campylobacter isolates from chicken meat in Mexico were C. coli.

Economic and Public health significance of Campylobacter isolates.

It has been estimated that from 24 to 61 million episodes of food borne illness occur per year in the United States, resulting in a 5 to 17 billion dollar loss in productivity **(IFT, 1986).** The handling of raw chicken, eating of under cooked or raw chicken or cross-contamination of raw or cooked foods could resulting

in acute Campylobacter enterocolitis in human throughout the world **(Aho and Hirn, 1988; Corry and Atabay, 2001; and Humphrey et al., 2001)**. The symptoms of such illness in humans is not easily distinguished from the other infections caused by another pathogens **(Adams and Moss, 1995)**. The incubation period is 2-7 days and the common clinical symptoms of enteropathogenic infection with C. jejuni and C. coli are abdominal pain, fever, and diarrhoea, sometimes accompanied by vomiting. Diarrhoea may be profuse, watery or alternatively bloody, leading to dysentry-like syndrome **(Walker et al., 1986; and ICMSF , 1996)**.

Concerning toxin production , both C. jejuni and C. coli produce an heat-labile cytotonic enterotoxin **(McCardell et al., 1986)**. In recent study, **Lee et al., (2000)** demonstrated a new heat-stable cytotoxin from C. jejuni that can resist heating at 100°C for 30 minutes. Moreover, over 70% of C. jejuni and C. coli strains also produce a cytotoxin **(Johnson and Lior, 1986; and Walker et al., 1986)**. Generally, the 4 major virulence factors recognized in this enteric pathogens are motility, adherence, invasion and toxin production **(Florin and Antillon, 1992; and Wassenaar, 1997)**.

From the results achieved in this study it could be concluded that fresh and frozen retail chicken carcases as well as roasted chickens marketed in Mansoura City have been shown to harbour Campylobacter jejuni and C. coli, and that may represent a potential helath hazard for humans.

Therefore, the following measures should be carried out to avoid or minimize the infection of human with such organisms: (i) application of hygienic measures during breeding of poultry for the production of chicken free from Campylobacter or for minimizing the colonizing strains ; (ii) hygienic rules of slaughter and poultry processing must be rigorously observed ; (iii) hygienic precautions to prevent cross-contamination of cooked food from raw poultry; (iv) implementation of good cooking techniques, also ; (v) education of food handlers and information of consumers about the risk associated with consumption of poultry and how to control it.

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