

Full Length Research Paper

Incidence of zoonotic *Campylobacter jejuni* in fast meal meat, grill chickens and symptomatic Egyptians

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Campylobacter jejuni is one of the most important foodborne gastroenteric zoonosis. Most strains of *C. jejuni* produce a toxin (cytolethal distending toxin) that hinders the cells from dividing and produces diffuse bloody edematous exudative enteritis. The common routes of transmission are fecal-oral, person-to-person and the eating of raw or undercooked chickens or meat. This study recognizes the incidence of zoonotic *C. jejuni* in under cooked chickens and meat meals along with persons in contact. We examined 640 grilled chickens and 733 fast meat meals, plus 93 of symptomatic consumers and handlers were collected from five Egyptian governorates (Fayuom, Cairo, Qaluobia, Bin-suef and Assuit) from different restaurants through culture-based methods for detection of *Campylobacter* motility. Also, molecular tools were used for genetic amplification by PCR using specific primers of *hipO* gene. Contamination with *C. jejuni* was recorded in 21.5% in chickens (16.6% in grill tissues and 26.2% in raw visceral organs) and 16% in fast meat meals (18.2% Offal, 15.2% Sausages, 20.4% Hamburger, 13.2% Kofta and 14.5% Shawarma), plus 19.4% in Egyptian personnel's (25.8% in handlers and 19.4% in symptomatic consumers). The polymerase chain reaction (PCR) showed identical fingerprints of *Campylobacter parvum* at 344 bp, signifying the high possibilities of zoonotic hazards. Dissimilar incidence of chickens, meat and humans were verified with reference to different governorates, but Assuit recorded higher percentages sequence to hot weather. The collected documents in this study can offer a base for the progress of public health requisites for advances in food safety measures.

Key words: *Campylobacter jejuni*, chickens, fast meat meals, consumers, polymerase chain reaction (PCR), Egypt.

INTRODUCTION

Campylobacter jejuni is gram-negative worldwide opportunistic bacteria, inducing one of the most notifiable gastroenteric foodborne zoonosis, due to the superior

levels of human consumption. *C. jejuni* affecting about 2.4 million people, with up to 15% of all human diarrheal cases every year (Marler, 2015). It has been confirmed in

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Table 1. Collects of different chicken meat samples into five regions.

Governorates	Grill chickens			Fast meat meals					Symptomatic in contact			
	Tissues	Visceral organs	T	Shawarma	Kofta	Hamburger	Sausages	Offals & liver	T	Consumers	Food handlers	T
Total samples	457	183	640	165	144	103	178	143	733	62	31	93
Fayuom	R97	40	137	36	35	24	39	33	167	16	7	23
Cairo	116	54	170	47	41	31	46	39	204	21	10	31
Qaluobia	82	35	117	34	38	25	38	35	170	12	5	17
Bin-suef	85	28	113	27	21	12	31	26	117	8	5	13
Assuit	77	26	103	21	9	11	24	10	75	5	4	9

various animal reservoirs, but poultry and their products have been recognized as the main source especially free-range ones (Humphrey et al., 2007). Most strains of *C. jejuni* have opportunistic characters and produce a cholera-like enterotoxin that hinders the cells from dividing, simulate watery diarrhea, fever and abdominal cramping (USDA, 2008). Bacterial infection is the main cause of disease of Guillian Barre Syndrome (GBS), so the most important human complication as acute demylenating disease of peripheral nervous system, paralysis of the limbs which lasts for several weeks, also, include toxic megacolon, dehydration and sepsis specially in children (<1 year of age) and immune-compromised patients (Allos, 2001; Yuki, 2001; Butzler, 2004).

Consumption or even handling of polluted raw or under cooked chickens or meat mostly lead to acute diarrhea (Yazdanpanah et al., 2000). Human incubation period is usually 2 to 5 days and untreated persons may shed the organisms for as long as 7 weeks (Heymann, 2004). In 2013, the UK's Food Standards Agency (Wagenaar et al., 2013) warned that two-thirds of all raw chicken bought from UK shops was contaminated with *Campylobacter*, affecting an estimated half a million people annually and killing approximately 100%, because of the "improper handling of foods by consumers and food service employees Wagenaar et al. (2013). Wadl et al. (2010) assurance that *C. jejuni* having different persistence and growth rate within meats that exposed to dissimilar cooked methods.

Polymerase chain reaction (PCR) targeting *hipO* gene was used previously for identification of *C. jejuni* in chickens, meat and human samples (Khalifa et al., 2013). The first *Campylobacter* genome to be sequenced was *C. jejuni* by Parkhill et al. (2000). The aim of the current study is to identify the possible zoonotic hazard of *C. jejuni* through under cooked grill chickens and the common Egyptian fast meat meals (Shawarma, Kofta, Hamburger, Sausages and Offal) along with Egyptian consumers and handlers.

MATERIALS AND METHODS

The practical work was done in Zoonotic Diseases Department, National Research Center, Egypt, from July 2013 up to January

2014. Samples collection is specified in Table 1.

Chicken samples

A total of 640 chicken samples, 457 grill tissues (core portions) from different restaurants and 183 raw visceral organs from poultry stores were collected from four Egypt governorates (Fayuom, Cairo, Qaluobia, Bin-suef and Assuit).

Fast meat meals samples

A total of 733 quick meat meals samples were collected as 165 Shawarma, 144 Kofta, 103 Hamburger, 178 Sausages, 143 Offals and liver from the same governorates mentioned earlier.

Human samples

Stool samples were collected from 93 persons, 31 were chickens handlers employees, from different markets and restaurants, and 62 were symptomatic consumers with history of food poisoning from poultry origin collected from the governmental hospitals or health unites from the same governorates mentioned earlier (Table 1).

All samples were collected in each plastic bag within 2 h. Placed in a cool box and transported immediately to the Laboratory, where they were processed within 2 to 4 h. The processing varied according to the type of sample placed into tubes containing 3 ml physiological saline (0.85% NaCl) and left to stand for 5 to 10 min to suspend before further processing (Makela et al., 2009).

Isolation and identification

About 10 g of each sample was homogenized in sterile thiogluconate broth. Broth samples were incubated at 42°C for 48 h. Under microaerobic condition (5% O₂, 10% CO₂ and 85% N₂). A loopful of enrichment broth were plated on semisolid thiogluconate broth (Oxoid) and incubated in microaerophilic atmosphere at 25, 37 and 42°C for 48 to 72 h in accordance with Iraola et al. (2012).

Microscopic examination of suspected colonies of *Campylobacter* were stained with Gram's stain and identified under phase contrast microscope using (1000x) magnification power (Smibert, 1984). For detection of characteristic comma, S-shape and spiral motility characters of the isolated *Campylobacter* organisms and deep stab growth are typical growth ring test. According to Hald et al. (2008), suspected colonies plated onto blood agar plates. *Campylobacter* isolates were subcultured and identified by biochemical tests (Acha

Table 2. Results of biochemical tests of *C. jejuni* isolated from food samples and human in contact.

Test isolate	Oxidase reduction	Catalase reduction	Motility	H ₂ S production	Growth in 1% glycine	Growth in 3.5% NaCl	Heat tolerance to 42°C	Sodium hippurate hydrolysis	Sensitive to nalidixic acid	Sensitive to cephalothin
<i>C. jejuni</i>	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	S	R

+ve= positive result; -ve= negative result; S= sensitive result; R= rare.

Table 3. Different results of isolated percentages of chicken, meat and human samples reference to varied governorates.

Governorates	Samples	Fayuom (%)	Cairo (%)	Qaluobia (%)	Bin-suef (%)	Assuit (%)	Total (%)
Chickens	Grill tissues	16 / 97 (16.5)	21/116 (18.1)	13/82 (15.9)	11/85 (12.9)	15/77 (19.5)	76/457 (16.6)
	Raw visceral organs	11/40 (27.5)	17/54 (31.5)	7/35 (20)	6/28 (21.4)	7/26 (26.9)	48/183 (26.2)
	Total	27/137 (19.7)	38/170 (22.4)	20/117 (17.1)	17/113 (15)	22/103 (21.4)	124/640 (19.4)
Fast meat meals	Shawarma	6/36 (16.7)	7/47 (14.9)	5/34 (14.7)	2/27 (7)	4/21 (19)	24/165 (14.5)
	Kofta	4/35 (11.4)	4/41 (9.8)	5/38 (5.7)	3/21 (14.3)	3/9 (33.4)	19/144 (13.2)
	Hamburger	5/24 (20.8)	7/31 (22.6)	3/25 (12)	2/12 (16.7)	4/11 (36.4)	21/103 (20.4)
	Sausages	6/39 (15.4)	7/46 (15.2)	5/38 (13.2)	4/31 (12.9)	5/24 (20.8)	27/178 (15.2)
	Offals & liver	7/33 (21.2)	5/39 (12.8)	8/35 (22.9)	3/26 (11.5)	3/10 (30)	26/143 (18.2)
	Total	28/167 (16.8)	30/204 (14.7)	26/170 (15.3)	14/117 (12)	19/75 (25.3)	117/733 (16)
Symptomatic individuals	Consumers	3/16 (18.8)	4/21 (19)	2/12 (16.7)	1/8 (12.5)	2/5 (40)	12/62 (19.4)
	Food handlers	2/7 (28.6)	1/10 (10)	1/5 (20)	2/5 (40)	2/4 (50)	8/31 (25.8)
	Total	5/23 (21.7)	5/31 (16.1)	3/17 (17.6)	3/13 (23.1)	4/9 (44.4)	20/93 (21.5)

et al., 2004).

For DNA extraction, colonies were suspended in 500 µl of PBS, pH 7.2, washed 3 times in PBS. The cell suspension was centrifuged for 10 min at 800 xg, then the supernatant was discarded carefully and the pellet was dried and stored at -20°C till use.

Molecular characterization of *C. jejuni*

Isolation of DNA

For extraction of DNA, bacterial pellets were re-suspended in 200 µl PBS and DNA was extracted using QIAamp DNA Mini Kit (Cat No.51304, Qiagen) according to manufacturer's instructions. The DNA pellet was dissolved in 50 µl of elution buffer. Extraction of genomic DNA from *C. jejuni* as mentioned earlier for use as a positive control.

DNA amplification reaction

PCR reaction contained 5 µl template DNA and 1 µl *hipO* primers (0.3 µM) (Wang et al., 2002). CJF (ACTTCTTTATTGCTTGCTGC) and CJR (GCCACAACAAGTAAAGAAGC) were performed in a total reaction volume of 50 µl containing 25 µl Taq PCR master mix (ViVantis Co., Malaysia). Thermocycler conditions were 95°C for 6 min, followed by 30 cycles of 94°C for 30 s, 59°C for 30 s and 72°C for 30 s and finally 72°C for 7 min. Positive controls was incorporated with each set of test samples and subjected to PCR assays. The PCR amplified products were loaded onto gels of 1.5%

agarose gel and stained with RedSafe™ Nucleic Acid Staining Solution Cat. No. 21141, iNtRON Co.) and visualized under UV trans-illuminator against 100 bp DNA ladder (Khalifa et al., 2013).

RESULTS

A total of 640 grill chickens and 733 fast meat meals, plus 93 of symptomatic consumers and handlers were examined from five Egyptian governorates (Fayuom, Cairo, Qaluobia, Bin-suef and Assuit) from different restaurants (Table 1). Through culture-based methods, the biochemical estimation of *C. jejuni* carried out by deep stab growth, typical growth ring test on semisolid thiogluconate broth at 37°C under microaerophilic conditions (5% O₂, 10% CO₂ and 85% N₂), and examined after 24 h under phase contrast microscope (Table 2) for characteristic *Campylobacter* motility and identification of characteristic comma, S-shape. Also, growth colonies were observed onto blood agar plates.

Table 3 and Figures 1 and 2 represented *C. jejuni* contamination values; 21.5% in chickens (16.6 in grill tissues, 26.2 in raw visceral organs) and 16% in fast meat meals (18.2% Offal, 15.2% Sausages, 20.4% Hamburger, 13.2% Kofta and 14.5% Shawarma), plus 19.4% in Egyptian personnel's (25.8% in handlers and 19.4% in symptomatic consumers). Dissimilar incidence

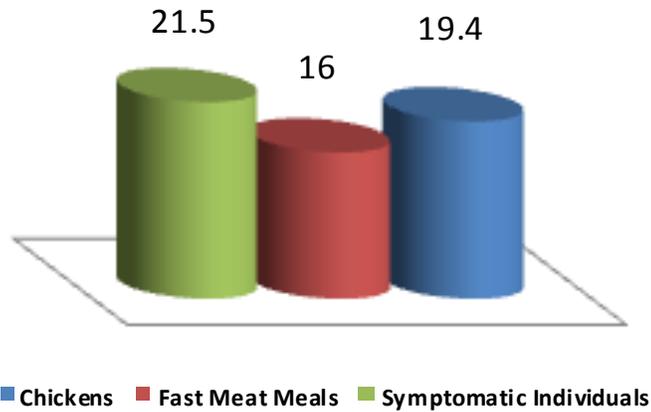


Figure 1. Total incidence of *C. jejuni* in chickens, meat meals and individuals reference to the overall studied governorates.

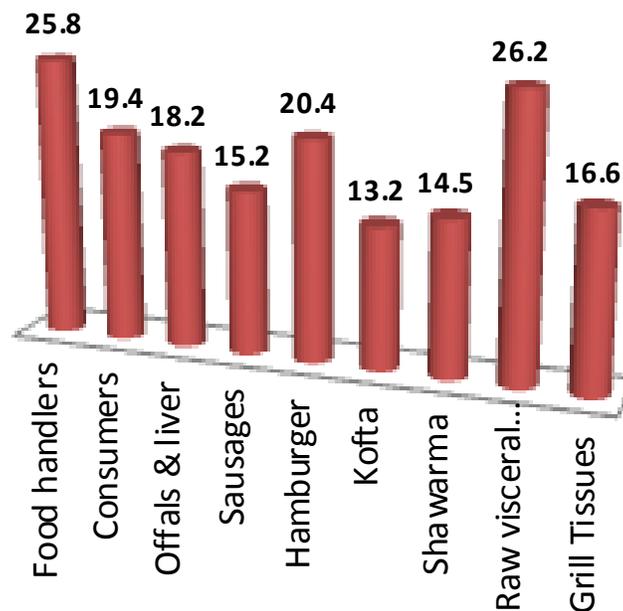


Figure 2. Overall percentages of *C. jejuni* in each food Organs.

of chickens, meat and humans were verified with reference to different governorates (Table 3 and Figure 3), Fayuom (21.7, 16.8 and 19.7), Cairo (16.1, 14.7 and 22.4), Qaluobia (17.6, 15.3 and 17.1), Bin-suef (23.1, 12 and 15) and Assuit (44.4, 25.3 and 21.4).

PCR using specific primers of *hipO* gene is in accordance with Wang et al. (2002). PCR confirmed all the bacteriologically positive isolates with a PCR product of 323 bp (Figure 4).

DISCUSSION

Chickens having up to 100% asymptomatic carriers of *C.*

jejuni in their intestinal tracts and may harbor up to 10^9 bacteria per 25 g, which rapidly spread among other chickens. This much exceeds the human infectious dose of about 10^3 bacteria (Humphrey et al., 2007). The present study identify overall positive chicken samples which harbor *C. jejuni* to be 19.4% (Table 3 and Figure 1) and might referred to the original intestinal contamination during bird evisceration (Moore et al., 2005). Our result was lower than that of Khalifa et al. (2013) (36%) and El-Tras et al. (2015) (23.5%); the prevalence differences can be attributed to isolation methods, sample types and size in addition to seasonal and regional variations (Allos, 2001; Shimaa et al., 2015). Varied values with different types of chicken samples were recoded (Figure 2).

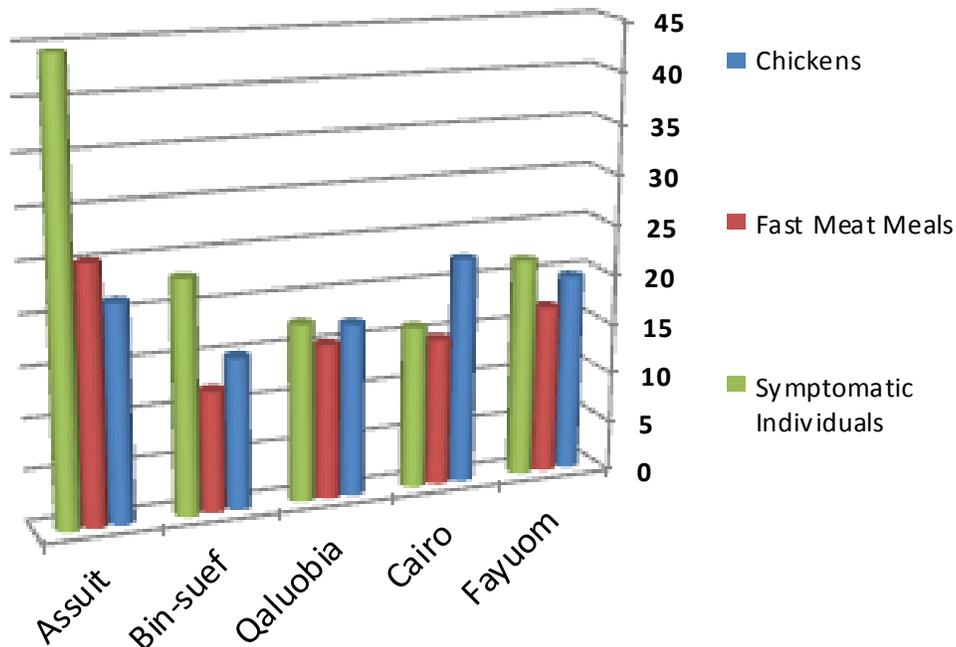


Figure 3. Total incidence of *C. jejuni* in chickens, meat meals and individuals reference to each studied governorates.

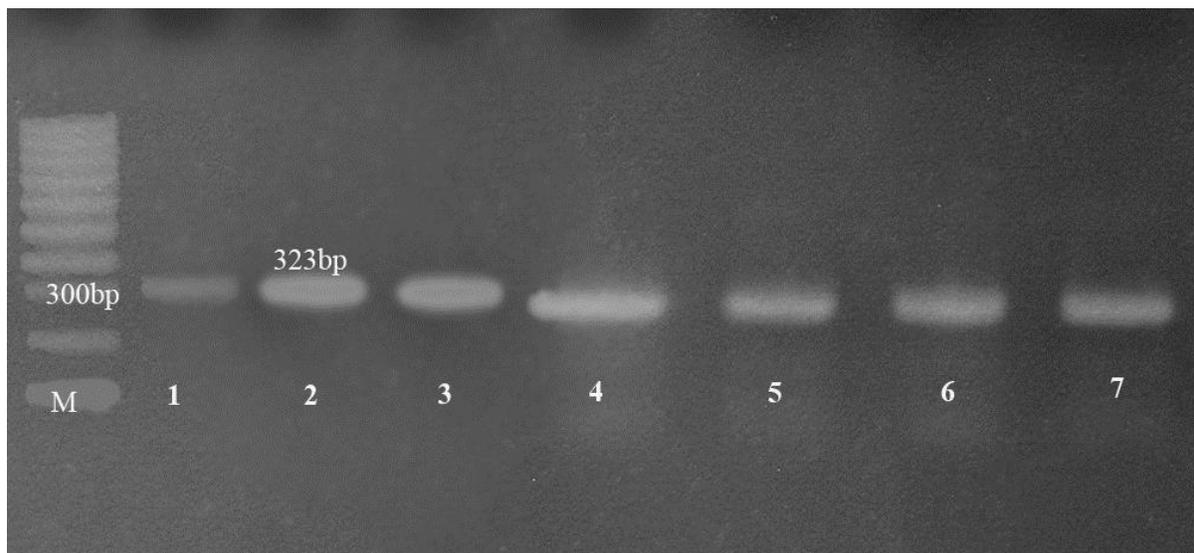


Figure 4. PCR amplification of the 323 bp products of DNA extracted from *C. jejuni*. Lane M: a 100 bp molecular size ladder. Lane 1, positive control; Lanes 2-4, are *C. jejuni* isolates from chicken samples, and 5-7, human samples respectively.

However, the raw offal and liver showed higher values (26.2%) than the barbeque chickens (16.6%). Visceral organs and grill tissues frequently polluted via either initial contamination from farm origin or pollution during processing via preparing utensils or food handlers. Also, most chicken farms do not have security fence to prevent penetration of other wildlife which are good carriers of

Campylobacter including rats, exotic birds and insects. Furthermore, poor hygiene measures during process of slaughter possibly contaminate poultry carcasses. USDA researchers confirmed that the most retail chicken is contaminated with *C. jejuni* with an isolation rate of 98% for trade chicken meat; where *C. jejuni* counts often exceed 10^3 per 100 g, skin and offal have particularly

high levels of contamination (USDA, 2008). Also, dissimilar incidence was recorded for *C. jejuni* concerning under-cooked barbequed chickens which may be due to the varieties of treatment methods which differ in temperature degrees and pH or spices (Wang et al., 2013). However, socio-economic difference via different governorates was a non-negligible factor represented in different hygienic measures applied during preparation or cooking. Cleaning and disinfection of water-line between farms flocks may help to reduce the risk of chicken *Campylobacter* colonization (Newell and Fearnley, 2003).

The contaminated or under cooked quick meat meals are one of the major cause of *Campylobacter enteritis* in human due to *C. jejuni* (Heymann, 2004). Due to the varieties of cooking methods which differ in temperature degrees and courses, in addition to varied pH and salt concentration. So, the temperature within core parts along with short cooking time could be sustain the favorable media for *Campylobacter* persistence and survival and may be multiplied with lowering heating degrees (Haan et al., 2010). The current study established this concept through verified dissimilar incidence of *C. jejuni* in different types of examined quick meat meals; offal and liver sausage, hamburger, kofta and shawarma were recorded with varied values: 18.2, 15.2, 20.4, 13.2 and 14.5%, respectively, while the overall rate of isolation was 16% (Table 3 and Figure 1). This result was lower than that recorded in quick meat meals samples; 59% by Ledergerber et al. (2003), 40% by Altekruse et al. (1999), 54% by Ekdahl and Andersson (2004) and 20% by Lutgen et al. (2009). The difference may be related to the varied types of processed meat and that Egyptian consumers usually prefer the well done meat.

The current study approve overall (21.5%) zoonotic hazard within Egyptian individuals. The handler employees recorded 25.8% higher percentages than the symptomatic consumers (19.4%) (Table 3 and Figure 2), denoting that human manipulate and contact withdraw chickens or contaminated meat represent higher risk than consuming cooked meals. Shedder poultry and the polluted ones during slaughter or carcass dressing possible maximize infect of handlers and in contact especially those having skin abrasions. Furthermore, poor hygienic measures exploit the common routes of transmission from polluted chickens or droplets of polluted water and spread by fecal-oral, person-to-person (Barakat et al., 2015). In contrast, Shima et al. (2015) detected higher infection percentage within Egyptian consumers than handlers. The difference in results may be attributed to the number of examined human samples and/or the food varieties. However, study of 156 human and 682 chicken strains validated equal strains in 70.7% of families of human isolates from diarrhoeal and non-diarrhoeal cases were identical to a household chicken isolate (Oberhelman et al., 2003). Other studies were in line with our results, where a survey in Cairo, Egypt

determined the prevalence, seasonality, and household risk factors for *Campylobacter*-associated diarrhea in children; *Campylobacter* species were more prevalent when associated with keeping fowl in the home (Pazzaglia et al., 1993). The organism is isolated from infants and young adults more frequently than from persons in other age groups (CDC, 2014). The high occurrence of *C. jejuni* in ready to eat quick meat meals in Egyptian restaurants, points toward the supervision defects of Egyptian health authorities concerning the suspected carrier shedders from at risk groups including meat and poultry handlers, farmers, restaurants staff, butchers and transported workers. Also, the results ensure the leak of sanitary measures during slaughtering or preparing poultries and animals that pollute products over the permissible limit than that of about (10^3) *C. jejuni* bacteria.

The benefit of salt and spices not only improve the edible test, but minimize bacterial growth within the permissible limit sequence to their bacteriostatic effects. Thus, salt and spices must be industrial applied during chilling storing phase for 2 h prior to meat or poultry cooking. Also, adding natural and lemon juice with salt concentration up to 7% of bacteriostatic action (Pham et al., 2010). However, *C. jejuni* polluted fast meat meals or chickens reflect either initial bacterial contamination or improper application of naturals and spices all through chilling store phase (Coker et al., 2002; Mauer et al., 2006).

Different governorates had recorded clear dissimilar values of *C. jejuni* contaminated chicken and meat samples (Table 3 and Figure 3). This variation may be due to warm or cold weather in addition to population behaviors (Lengerh et al., 2013). Higher temperature and humidity enhance *Campylobacter* growth (Refregier-Petton et al., 2001; Bouwknegt et al., 2004). The reason is still debated, but may indicate a possible relationship between temperature and *Campylobacter* survival and transmission as stated by Patrick et al. (2004). Also, insects frequently involved in summer season may be an important source of *Campylobacter* via mechanical transmission, where flies, cockroaches and other insects passed through the ventilation system into the chickens' house and the invasion of insects correlated with the outdoor temperature (Hald et al., 2008). So, in the current study, Assuit governorate with hot weather recorded the higher values of both human exposure and contaminated chicken and meat samples, than the others moderate weather Egyptian governorates (Figure 3).

Culture-based methods are time consuming and expensive, requiring filtration, selective enrichment, isolation and biochemical confirmation (Table 2). The application of molecular tools, such as PCR (Figure 4), may help to avoid some of the limitations of current methods, where the *hipO* gene is specific for *C. jejuni* strains (Sinha et al., 2004).

This study aimed to identify the zoonotic *C. jejuni*,

through culture-based methods and genetic characteristics of collected isolates from Egyptian under cooked ready-to-eat chickens and fast meat meals incriminated in high infection rate within Egyptians consumers and handlers' employees', reflect on advances in food safety events and provides background for the design of firm efficient control strategies.

Conflict of interest

The authors have not declared any conflict of interest.

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