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Incidence of *Campylobacter* species in wholesale chicken carcasses and chicken meat products in Assiut city, Egypt

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Abstract

Campylobacter was investigated in chicken meat and chicken meat product harvested in Assiut. The conventional isolation method were used for isolation of campylobacter and further confirmed by polymerase chain reaction (PCR). Out of ninety samples of fresh, frozen whole chicken carcasses and chicken based products (30 samples each), 20/30 (66.7%) was positive for *Campylobacter* by conventional isolation method in each of examined fresh and frozen whole chicken carcasses. Two samples were isolated from chicken nuggets. *Campylobacter* couldn't be detected in chicken burger. Eighty percent (16/20) was positive for *campylobacter* genus by the simplex PCR in each of examined fresh and frozen whole chicken carcass. Equal frequencies of *C. jejuni* (87.5%) fourteen out of sixteen samples confirmed positive for *Campylobacter* genus by the simplex PCR) were noticed in each of examined fresh and frozen whole chicken carcasses, while *C. coli* detected at rate of 14/16(87.5%) and 12/16(75%), respectively by duplex PCR assay. Both of *C. jejuni* and *C. coli* were detected and confirmed by duplex PCR in the two positive samples of chicken nuggets. According to this study, it appears that the incidence of *C. jejuni* and *C. coli* almost equal in the examined samples. Also nearly the same rate of occurrence of campylobacter in fresh and frozen chicken carcass, but in chicken meat products is very low.

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Introduction

Consumption of chicken meat and chicken meat products is increased worldwide which may be attributed to the recommendations of healthy nutrition based on high protein content and PUFA, its low price, simplicity to be prepared and ease to get in retail markets. However, raw and under cooked poultry and poultry products are the major sources of campylobacteriosis (Adzitey *et al*., 2011).

Campylobacters are microaerophilic, Gram negative, small vibrioid or spiral-shaped cells with rapid, darting, reciprocating motility. *Campylobacter* species is main cause of bacterial gastrointestinal; campylobacteriosis; disease. It produces diarrhea, sometimes dysentery syndrome, mostly including cramps, fever and pain in the developing countries. *Campylobacter jejuni* and *C. coli* are accountable for the illness. *C. jejuni* infections cause a latent autoimmune on the nerves of the legs after a surgical procedure of the abdomen. It is known as an acute idiopathic demyelinating polyneuropathy (AIDP), i.e. Guillian-Barré Syndrome ended with respiratory failure. The infection may accompanied by haemolytic uraemic syndrome. (EFSA report, 2010; Sammarco *et al*., 2010).

Campylobacter naturally colonize the chicken gut. Thus, the intestinal tract of chicken supplies a reservoir for *Campylobacter* which may spread via fecal material at farm or during processing. Thus, reduction of contamination of raw poultry has a great effect in declining the occurrence of illness. Contaminations happen since the birds on farms till in poultry-slaughtering plants. Routine actions on the farm, such as feed withdrawal, handling and transportation of poultry have a reported effect on *Campylobacter* levels at the processing plant. At the plant, all processes from defeathering till carcass chilling have been reported to cause cross-contamination to poultry carcasses. Carcass washings and the use of processing aids have been revealed to decrease *Campylobacter* levels in carcass rinse (Keener *et al*., 2004).

Molecular methods used for confirmation of detected *Campylobacter* species and to differentiate between *C. jejuni* and *C. coli*, while the detection and discrimination of *C. jejuni* and *C. coli* is considered difficult because it only depends on a single phenotypic test stand on the hydrolysis of hippurate (Steinhauserova *et al*., 2001).

Most studies conducted on *campylobacter* spp. isolation from chicken meat, but few researches were carried out on the relation of occurrence of campylobacter in chicken carcasses and its presence

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in chicken meat products. Thus the goal of this study was to verify the incidence of theromtolerant campylobacter species in retailed fresh, frozen chicken carcass and chicken products using conventional culture method and PCR based techniques.

Material and Methods

Collection of samples

A total of 90 samples of chicken carcasses and chicken products were collected from retail markets in Assiut city in Egypt from September 2014 to May 2015. These samples were split into 30 of each fresh and frozen chicken carcasses and 30 of chicken products of which 15 of chicken nuggets and 15 of chicken burger. All samples were transported to the laboratory within 1 hr.

Preparation and enrichment of chicken rinse sample

Carcass was placed in sterile polyethylene bags. The chicken carcasses were rinsed with 400 mL of buffered peptone water (Difco). A 25-mL test portion from carcass-rinse sample was added to 25 mL of Bolton selective enrichment broth contains Sodium Pyruvate, Sodium Metabisulphite and Ferrous Sulphate (FBP) Campylobacter growth supplement without blood incubated at 37°C for 4h, then at 42 for 48 h. (USDA, 2011).

Enrichment for chicken products

Ten grams of chicken meat products were homogenized in 90 ml of *Campylobacter* selective enrichment Bolton Broth contains FBP *campylobacter* growth supplement and incubated at 37°C for 4h, then at 42°C for 48 h.

Plating of enriched samples

A loopful of the incubated culture broth was streaked on modified Charcoal Cefoperazone Deoxycholate Agar (mCCDA) (Oxoid) and incubated under microaerophilic condition (5% O_2, 10%CO_2 and 85% N) at 42°C for 48 h.

Conventional isolation method

Presumptive identification of *Campylobacter* colonies was based on the colonial appearance and Gram-staining (Gram negative, highly motile rods with S-shaped or spiral morphology). The identification of isolates was based on characteristic reactions for hippurate hydrolysis, indoxylacetate hydrolysis and urease activity (Roberts and Greenwood, 2002).

DNA extraction

The strains were further identified by conventional PCR assays. For each *Campylobacter* strain, 1 ml of cells was centrifuged at 13,000 x g. for 5 min. The cell pellets were suspended in 1 ml of sterile phosphate buffer saline. Then the mixture was centrifuged at 12,500 x g. for 15 min. At last, the pelleted cells were used for DNA extraction. Genomic DNA from suspected *Campylobacter* strains was extracted using the Wizard genomic DNA purification kit (Promega, USA) as recommended by the manufactures. Purified DNA samples were stored at -20°C until use.

Simplex Genus-specific PCR assay

For *Campylobacter* genus PCR identification, Two primers were selected based on the primers previously described by Linton *et al*. (1996) (Table 1). All PCR reaction was performed in a final volume of 25 ul using 2 ul of extracted DNA as template. The reaction mixture contained 12.5 µl GoTaq® Green Master Mix (Promega, M7122), 1 µl of forward primer (C412F); 1 ul of reverse primer (C1228R) and 8 µl of Ultra-Pure DNase/RNase-Free distilled water (Gibco, Grand Island, NY, USA). The DNA amplification reactions were performed in thermal cycler (Techne Cyclgene, UK). The cycling conditions for PCR were as follows: intial denaturation at 95°C for 5 min; 40 cycles consisting of dsDNA denaturation at 95°C for 30 s, primer annealing at 56°C for 30 s, primer extension at 72°C for 30 s; final elongation at 72°C for 10 min. Amplified products were resolved in 1% agarose gel, stained with ethidium bromide, detected under a short-wavelength UV light source.

Duplex Species-specific conventional PCR

Two Primer sets described by Linton *et al.* (1997) (Table 1) were used to detect specific gene in Campylobacter species, including the hippuricase gene (hip) for *C. jejuni* and aspartokinase gene (asp) for *C. coli.* A duplex PCR was carried out for detection of *C. jejuni* and *C. coli* in each sample isolates. Amplicon sizes produced were of 735-bp and 500-bp, respectively.

Duplex PCR was performed with 5 µl of DNA sample, 25 ul of GoTaq Green Master Mix (Promega, M7122), 1 µM of each primer, and 15 ul of DNase / RNase free water in a final volume of 50 µl. The reactions were performed in a DNA thermocycler (Techne, cyclogene, UK). The m-PCR protocol consisted of the following steps: The initial denaturation step of 5 min at 95°C; 35 cycles consisting of dsDNA denaturation at 95°C for 30 s, primer annealing at 54°C for 30 s, primer extension at 72°C for 30 s; final elongation at 72°C for 10 min. The

Table 1. Primer sets for PCR amplification of the three particular genes of *Campylobacter* genus and species

Species	primer	sequence	Target gene	Size (bp)	Reference
Campylobacter genus	C412F C1228R	5'-GGATGACACTTTTCGGAGC-3' 5'-CATTGTAGCACGTGTGTC-3'	16S rRNA	816	Linton *et al.* (1996)
C. Jejuni	HIP400F HIP1134R	5'-GAAGAGGGTTTGGGTGGTG-3' 5'-AGCTAGCTTCGCATAATAACTTG-3'	hip O	735	Linton *et al.* (1997)
C.coli	CC18F CC519R	5'-GGTATGATTTCTACAAAGCGAG-3' 5'-ATAAAAGACTATCGTCGCGTG-3'	asp	500	Linton *et al.* (1997)

PCR products were subjected to electrophoresis in 1% (w/v) agarose gel, stained with ethidium bromide and photographed under UV transillumінator then documented with a gel documentation apparatus (Table 1).

Results

Conventional Isolation of Campylobacter

Overall, 42 (46.7%) out of 90 examined samples were contaminated with *Campylobacter.* Out of ninety samples of fresh, frozen whole chicken carcasses and chicken based products (30 samples each), 20/30 (66.7%) was positive for *Campylobacter* species by conventional isolation method in each of examined fresh and frozen whole chicken carcasses, and only 2/15(13.3%) in chicken nuggets. *Campylobacter* couldn't be detected in chicken burger (Table 2).

Identification of Campylobacter *by molecular methods*

The general prevelance of *Campylobacter* genus by the simplex PCR was 81% (34/42). The confirmed positive limit for *Campylobacter* by simplex PCR in each of examined fresh and frozen whole chicken carcasses was 16/20 (80%) with product size of 816 bp. Two positive samples of chicken nuggets also confirmed positive for *Campylobacter* by simplex PCR (Table 3 and Figure 1). Equal frequencies of *C. jejuni* ((87.5%) fourteen out of sixteen samples previously confirmed positive for *Campylobacter* genus by the simplex PCR)) were noticed in each of examined fresh and frozen whole chicken carcasses, while C. coli detected at rate of 14/16(87.5%) and 12/16(75%) by duplex PCR assay with products sizes of 735 and 500 bp, respectively. Both of *C. jejuni* and *C. coli* were detected and differentiated by the duplex PCR in each of the previously two confirmed positive samples for *Campylobacter* of chicken nuggets by the simplex PCR (Table 3 and Figure 2).

Analysis by molecular method was more accurate

Table 2. Incidence of *Campylobacter* genus in the examined samples by conventional method

Samples		Positive samples for *Campylobacter* genus	
Type	No.	No.	%
Fresh	30	20	66.7%
Frozen	30	20	66.7%
Chicken burger	15	0	0
Chicken nuggets	15	2	13.3%
Total	90	42	46.7%

than the conventional method. 20/30 of each of the examined fresh and frozen carcasses was confirmed positive for *Campylobacter* by the conventional method, while only16 samples were confirmed positive out of the twenty by the simplex PCR.

Discussion

Whole carcass rinse is used for more prediction of *Campylobacter* as reported by Jorgensen *et al.* (2002) who found that carcass rinse is used for isolation as it improves the detection of the *Campylobacter* spp. in the whole carcass. Often, if one part of the chicken has been contaminated all parts and even the inner parts of the chicken could be easily get contaminated, most likely due to cross contamination during processing (Nur Ilida and Faridah 2012).

In this study about 66.7% of each of fresh and frozen chicken carcasses was found contaminated with *Campylobacter* by the conventional isolation method from carcass rinse (Table2). This may originate from cross contamination from carcasses as the main reservoir of *Campylobacter* or from surrounding during slaughtering and subsequent processing. The similar result was reported by Tokumaru *et al.* (1991) in Japan, Harrison *et al.*,

Table 3. Detection of *Campylobacter* genus, *C.jejuni* and *C. coli* in the examined samples by PCR technique

Samples	*Campylobacter genus*		*C. jeuni*		*C. coli*	
	No.	%	No.	%	No.	%
Fresh	16/20	80%	14/16	87.5%	14/16	87.5%
Frozen	16/20	80%	14/16	87.5%	12/16	75%
Chicken burger	0	0	0		0	0
Chicken nuggets	2/2	100%	2/2	100%	2/2	100%
Total	34/42	81%	30/34	88%	28/34	82.4%

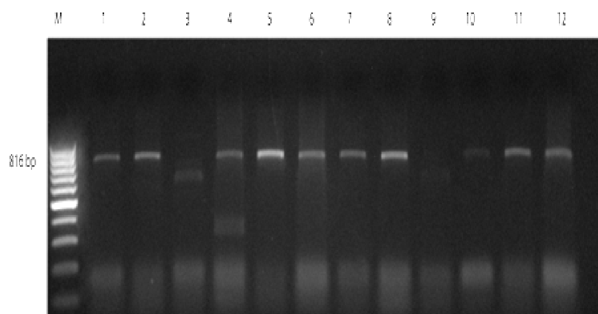

Figure1. 16S rRNA gene (816bp) specific for *Campylobacter* genus lanes 1,2,4-8,11,12, M 100 bp marker

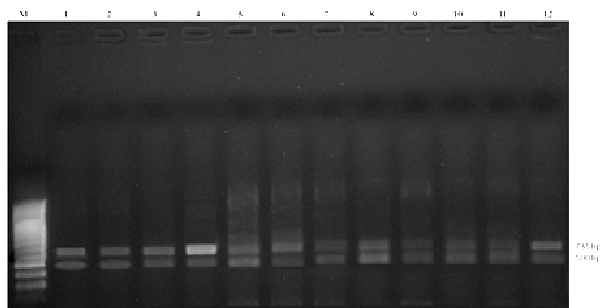

Figure 2. PCR products obtained by duplex PCR by using two primer sets derived from hippuricase (735bp) specific for *C. jejuni* and aspartokinase gene (500bp) specific for *C. coli,* (lane M : 100bp ladder, lane 1-12 positive for C. *jejuni* and *C. coli*).

(2001) in South Wales, and Sallam (2007) in japan who isolated *Campylobacter* spp. from raw chicken meat samples at the retail levels at rates 67.9%, 68%, and 64.7%, respectively . Their findings were referred to the cross-contamination of poultry carcasses during defeathering, evisceration, and carcass chillers (Harrison *et al*., 2001). Berrang and Dickens (2004) stated that feathers can be contaminated with feces during transport, and *Campylobacter* can be transferred to the skin during the plucking process. Sandberg *et al.* (2005) found that although freezing is recognized to reduce the *Campylobacter* contamination on broiler carcasses, 80% of the carcasses still contaminated with *Campylobacter* following 120 days of freezing. As well, it has been reported that thawing temperatures close to 0°C favor the survival of *Campylobacter* spp. (Georgsson *et al.*, 2006). The organism can survive and still infective through the refrigerated or frozen storage of poultry meat (Sampers *et al*., 2010).

Out of 15 chicken nuggets samples only 2 samples were found contaminated with *Campylobacter* by conventional (Table 2). This may attributed to cross contamination as frozen chicken-based products had undergone minimal cooking process prior to freezing which reduce risk of contamination with the organism. *Campylobacter* were not detected in chicken burger samples. This seems to be obtained by Nur Ilida and Faridah (2012) who could not isolate *C. jejuni* from 22 types of frozen chicken-based products. Also this result approximately resembles that stated by Lake *et al.* (2007) who mentioned that a study done by Joint Food Safety and Standard Group of United Kingdom (1996) found that 758 cooked-chilled chicken products analyzed were free from *Campylobacter.* Also, Lake *et al.* (2007) reported that Heating at 55°C and above, rapidly inactivates the organism.

The time consuming and fastidious characters of these methods has motivated research into molecular diagnostic approaches. Sodium hippurate hydrolysis is the most important reaction used to differentiate *Campylobacter jejuni* and *Campylobacter coli.* Several researchers have used multiplex PCR for the discovery of *Campylobacter* (Aquino *et al*., 2002; Klena *et al.*, 2004; LaGier *et a*l., 2004).

Molecular methods using simplex PCR assay used for detection and confirmation of Campylobacter genus using 16S rRNA gene at 816 bp (Linton *et al*., 1996) and duplex PCR was used to differentiate between *C. jejuni* and *C. coli* using hip. gene for *C. jejuni* and asp. gene for *C. coli* (Linton *et al*., 1997) giving 735 bp and 500 bp products,

respectively. Eighty percent (16/20) of fresh and frozen chicken carcass campylobacter positive samples by conventional methods were confirmed by PCR technique (Table 3 and Figure 1). This finding is almost in agreement with that reported by Englen and Fedorka-Cray (2002) who ended in that 89.5% (119/133) of Campylobacter strains from poultry carcass rinse were also matching when tested by commercial method and standard PCR. However, no difference was found between molecular methods and conventional culture technique for Campylobacter detection in chicken nuggets which in parallel with that reported by Mateo *et al.* (2005).

C. jejuni and *C. coli* were isolated from fresh chicken carcass and at the same rate (14/16(87.5%)), but *C. jejuni* isolated at higher rate (14/16(87.5%)) from frozen chicken carcass than *C. coli* (12/16(75%)). (Table 3 and Figure 2) for the reason that *C. jejuni* can survive high and low temperature, low pH and dry conditions (El-Shibiny *et al*., 2009). *C. jejuni* and *C. coli* were confirmed by duplex PCR in the previously detected two positive samples of chicken nuggets by the conventional method. This may for the reason that *Campylobacter* survives freezing and thawing successfully and continue to be a hazard for the consumers. That highlights the need for hygiene actions to reduce the threat in subsequent steps of heat processing (Stoyanchev *et al.*, 2007).

The findings of this study confirm that *Campylobacter* genus was found at the same rate in fresh and frozen chicken carcass by both conventional and molecular methods (Table 2 and 3). There was a very low contamination rate with the organism in the examined chicken nuggets samples, while chicken burger samples were not contaminated. This implies that *Campylobacter* may be not suppressed by freezing, but it is affected by heating. The contamination rate with *C. jejuni* and *C. coli* was almost equal in the examined samples. Slight difference was found between molecular techniques and conventional culture method for *Campylobacter* detection, but PCR-based assays are faster, more specific, sensitive and of prime interest for recognition and verification of *Campylobacter* species. So, PCR-based assays are dependable substitute to conventional culture method for the discovery of the objective microorganisms.

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