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Molecular and conventional methods for detection of Campylobacter spp. in retail poultry cuts, offal and chicken products

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A B S T R A C T

The goal of this study is to determine the prevalence of *Campylobacter spp.* in some poultry products by using both conventional and molecular methods. Two hundred chicken product samples were collected from various supermarkets at Menofia government (25g of each) of chicken breast with skin & without skin, thigh with skin &without skin, liver, gizzard, nuggets and luncheon. Most samples were contaminated with *Campylobacter spp.* except chicken luncheon. Chicken liver showed the highest contamination (92%) followed by gizzard(80%), thigh with skin (72%), thigh without skin (68%), breast with skin (60%) and breast without skin (48%). The lowest contamination was Chicken nuggets of 20 %. *Campylobacter.jejuni* (33.1%) was the most isolated serotype, and also followed by *C.coli*(16.6%), *C.lari* (6.9%), *C.cinaedi*(1.7%) , *C.butzieri* (1.7%) and *C.upsaliens*(1.1%) . *hipO* gene was detected in isolated *C. jejuni*. Virulence genes *cdtA, cdtB* and *cdt C* , *cdtA* and *cdtC* ,*cdtB* and *cdtC* ,*CdtA, CdtB* and *CdtC* were 30% , 15%, 25%, 10% ,15 and 5 of isolated strains of *C.jejuni*, respectively. Finally the application of molecular methods is more favorable than conventional methods in detection of *Campylobacter* serotypes.

Key words: *Chicken meat; Campylobacter jejuni ; liver; nuggets; PCR.*

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1. INTRODUCTION

Chicken meat industry is the biggest supplier of acceptable animal protein with high meat yield, low shrinkage in cooking and great source of amino acids, vitamins and minerals (Oulkeir, et al., 2017).

Campylobacter has been recovered from chicken carcasses, poultry meat parts and equipments in processing plants worldwide García-Sánchez et al., (2017).

Campylobacter is a zoonotic pathogen and is the main cause of human bacterial gastroenteritis in the world (Humphrey and O'Brien, 2007 and Tam and Rodrigues, 2012). The most often reported pathogenic species is *C. jejuni* accounting for more than 90% of the cases, followed by *C. coli* representing 7% of the infections, with the rest of cases being mainly *C. lari* and *C. fetus* (Moore and Corcoran, 2005).Human *C. jejuni* and *C. coli* infections do not differ regarding clinical symptoms and duration of illness. However, patients infected with *c. coli* tend to be older than those with *C. Jejuni* (Karenlampi and Rautelin, 2007).The incubation period is two to five days, and the infection results in an acute self-limiting gastrointestinal illness typically resolved in one week, characterized by mild to severe watery/bloody diarrhea, fever, nausea, malaise and abdominal pain (Blaser, 1997).In developed countries most of the human cases occur from late spring until summer (Kovats and Edwards, 2005).Mortality rate is poorly defined but low, with deaths normally confined to immuno-compromised patients or those suffering from another severe disease such as bowel cancer (Allos, 2001).There is considerable epidemiological evidence that the most significant risk factor associated with human *Campylobacter* infection is the presence of this organism in chicken (Sheppard, Dallas *et al*., 2009).In addition to direct carcass contamination, intestinal contents contaminate machines, working surfaces, protective clothing and employee's hands increasing the opportunity for cross-contamination of

Campylobacter-free carcasses (FAO and WHO, 2002).

The identification and discrimination of *C. jejuni* and *C. coli* is considered problematic because it described as an alternative to the inaccurate, time consuming, biochemical phenotypic methods (LaGier *et al.*, 2004). A number of conventional PCR assays targeting a variety of genes such as hipO, glyA, cadi, ceuE and map A have been documented (On and Jordan, 2003). So, the goal of this study is identification of *Campylobacter* spps. by molecular and conventional methods in some chicken cuts, offal and chicken products.

2. MATERIAL AND METHODS

2.1. Collection and preparation of samples

A total of 200 different chicken product samples were collected from random supermarkets at El-Menoufia government. Samples consisted of chilled breast with skin & without skin, thigh with skin & without skin, liver, gizzard, frozen chicken nuggets and luncheon. Samples were individually wrapped and stored in coolers (±4C°) and then transported to the laboratory soon without undue delay.

2.2. Assessment *of Campylobacter* count:

2.2.1. Preparation of samples in enrichment *broth*

Twenty five grams of each sample were aseptically transferred to a sterile blender containing 225 ml of Preston enrichment broth for homogenization then enriched (Sallam, 2001).

2.2.2. Isolation in selective media

For isolation of *Campylobacter* species, the collected samples in Preston enrichment broth were incubated at 42ºC for 24-48 hours with less than 1 cm of headspace left in the culture vessel with tightly capped lids (Oxoid, 2006). After enrichment, 0.1 ml of the broth was streaked onto modified campylobacter selective agar base Cefoperazone Charcoal Desoxycholate Agar; mCCDA containing CCDA selective supplement. The plates were then incubated at 42ºC in darkness for 48 hours under microaerophilic conditions (5% O2, 10% CO2 and 85% N2) using CampyGen sachets (Vandepitte and verhaegen, 2003).

only depends on a single phenotypic test based on the hydrolysis of hippurate (Steinhauserova *et al.*, 2001). Therefore, molecular identification methods have been

2.3. Identification of Campylobacter:

2.3.1. Morphological identification:

The preliminary identified Campylobacter species were further subjected to Gram staining, testing of motility. (OIE, 2008).

2.3.2. *Biochemical identification:*

Growth at 25ºC and 41.5ºC, catalase test, oxidase test, susceptibility to nalidixic acid and cephalothin and rapid hippurate hydrolysis test (OIE, 2008).

2.3.3.Serological identification:

It was carried out according to Oyarzabal *et al.* (2007). Positive samples were serologically examined by Latex Agglutination Test

2.3.4 Molecular identification :

Polymerase Chain Reaction (PCR) was done to confirm the previously identified isolates of *Campylobacter jejuni* and examined the strains for the occurrence of virulence genes and it was done by multiplex PCR for cytological distending toxins cdtA(631 bp), cdtB (714 bp) and cdtC (524 bp).

2.3 4.1 DNA Extraction using QIA amp kit (Ehsannejad et al., 2015):

Boiling extraction method described by was used to extract DNA from Campylobacter

2.3 4.2. DNA amplification:

2.3 4.3. Amplification reaction of hipOgene(Wang et al., 2004):

The amplification was performed on a Thermal Cycler (Master cycler, Eppendorf, Hamburg, Germany

2.3 4.4. Amplification of the selected virulence genes (Carvalho et al., 2013):

Actually, 40 µl of PCR mixture were used. All reactions contained appropriate concentration of 3 primer sets, 0.2 mM each of dATP, dCTP, dGTP and dTTP, 1 × Ex *Taq*DNA polymerase buffer, and 1.0 U of Ex *Taq*DNA polymerase in a 40-mL reaction volume

2.5.3. Primer sequences used for PCR identification system:

Target gene	Oligonucleotide sequence (5′ → 3′)	Product size (bp)	References
hipO (F)	5′ TATACCGGTAAGGAGTGCTGGAG ′3		Wang *et al.* (2002)
hipO (R)	5′ ATCAATTAACCTTCGAGCACCG ′3	650	
cdtA (F)	5′ AGGACTTGAACCTACTTTTC ′3		
cdtA (R)	5′ AGGTGGAGTAGTTAAAAAC ′3	631	Asakura *et al.* (2008)
cdtB (F)	5′ ATCTTTTAACCTTGCTTTTGC ′3		
cdtB (R)	5′ GCAAGCATTAAAATCGCAGC ′3	714	
cdtC (F)	5′ TTTAGCCTTTGCAACTCCTA ′3		
cdtC (R)	5′ AAGGGGTAGCAGCTGTTAA ′3	524	

3. RESULTS

As shown in table (1) results revealed that the incidence of *Campylobacter spp.* was positive for all samples except chicken luncheon. The highest incidence was found in chicken liver and gizzard (80 %) followed by thigh with skin(72 %), thigh without skin (68 %), breast with skin (60 %) breast without skin (48 %).and the lowest one was chicken nuggets(20 %).

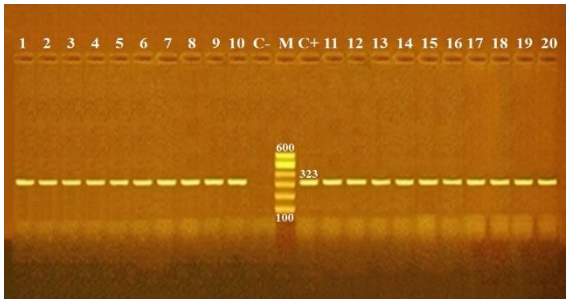
It is evident from results recorded in table (2), the incidence of *C. jejuni* , *C.coli* and *C.butzieri* were 28 %, 20 % and 12 % in breast with skin; *C. jejuni* , *C.coli* and *C.lari were* 24 %, 16 % and 8 % in breast without skin;*C. jejuni* , *C.coli* and *C.lari* and *C.cinaedi were* 40 %, 16 %, 8% and 8 % in thigh with skin;*C. jejuni* , *C.coli* , *C.lari* and *C.upsaliens* were 36 %, 12 %, 12 % and 8 % in thigh without skin; *C. jejuni* , *C.coli, C.lari* and *C.cinaedi* were 48 %, 20 %, 8 % and 4 % in liver. *C. jejuni* ; *C.coli* and *C.lari were* 44 %, 24 % and 12 % in gizzard and *C. jejuni* & *C.coli were* 12 % and 8 % in nuggets, respectively.

Table (1): Incidence of *Campylobacter* serotypes in the examined chicken product samples (n =25)

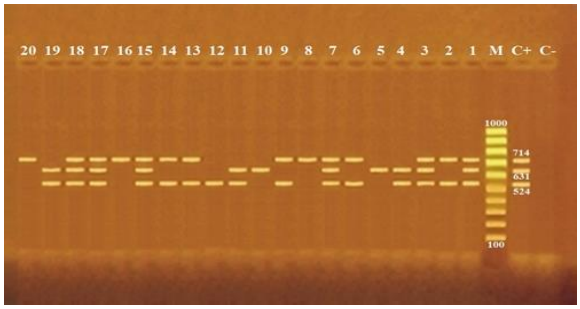
Chicken products	Positive samples	
	No.	Percentage %
Breast with skin	15	60
Breast without skin	12	48
Thigh with skin	18	72
Thigh without skin	17	68
liver	20	80
gizzard	20	80
Chicken nuggets	5	20
Chicken luncheon	0	0

% was calculated according to total number of examined samples.

Table (3) and photo (1 & 2) showed the Occurrence of virulence genes of *C. jejuni* strains isolated from all the examined samples of chicken products except luncheon. Virulence genes *cdtA*, *cdtB* and *cdt C* , *cdtA* and *cdtC* ,*cdtB*and *cdtC* ,*CdtA*, *CdtB* and *CdtC* were present in 30% , 15%, 25%, 10% ,15 and 5 of examined of strain of *c.jejuni* respectively.



Photograph (1):Agarose gel electrophoresis of PCR of *hipO* (323 bp) as confirmatory gene for detection of *Campylobacter jejuni*.
Lane M: 100 bp ladder as molecular size DNA marker.
Lane C+: Control positive for *hipO* gene.
Lane C-: Control negative.
Lanes from 1 to 20: Positive *Campylobacter jejuni* strains for*hipO* gene.



Photograph (2):Agarose gel electrophoresis of multiplex PCR for cytological distending toxins *cdtA* (631 bp), *cdtB* (714 bp) and *cdtC* (524 bp) as virulence genes for characterization of *Campylobacter jejuni.*
Lane M: 100 bp ladder as molecular size DNA marker.
Lane C+: Control positive *C. jejuni* for *cdtA, cdtB* and *cdtC* genes.
Lane C-: Control negative.
Lanes 1, 3, 7, 15, 17 & 18: Positive *C. jejuni* for *cdtA, cdtB* & *cdtC* genes.
Lanes 4, 11& 19: Positive *C. jejuni* strains for *cdtA* and *cdtC* genes.
Lanes 2, 6, 9, 13 & 14: Positive *C. jejuni* strains for *cdtB* and *cdtC* genes.
Lanes 5 & 10: Positive *C. jejuni* strains for *cdtA* gene.
Lanes 8, 16 & 20: Positive *C. jejuni* strains for *cdtB* gene.
Lane 12: Positive *C. jejuni* strain for *cdtC* gene.

Table (2) Serotyping of *Campylobacter* strains isolated from examined chicken product samples (n =25)

Chicken products	C.jejuni		C.coli		C.lari		C.cinaedi		C.upsaliens		C.butzieri	
	No.	%	No.	%	No	%	No.	%	No.	%	No.	%
Breast with skin	7	28	5	20	0	0	0	0	0	0	3	12
Breast without skin	6	24	4	16	2	8	0	0	0	0	0	0
Thigh with skin	10	40	4	16	2	8	2	8	0	0	0	0
Thigh without skin	9	36	3	12	3	12	0	0	2	8	0	0
Liver	12	48	5	20	2	8	1	4	0	0	0	0
Gizzard	11	44	6	24	3	12	0	0	0	0	0	0
Total	55	36.6	27	18	12	8	3	2	2	1.3	3	2

% was calculated according to total number of examined samples.

Table (3): Occurrence of virulence genes of C. jejuni strains isolated from the examined samples of chicken meat and giblets (n= 20).

Virulence genes	No.	%
cdtA, *cdtB* and *cdtC*	6	30
cdtA and *cdtC*	3	15
cdtB and *cdtC*	5	25
cdtA	2	10
cdtB	3	15
cdtC	1	5
Total	20	100

4. DISCUSSION

Chickens having up to 100% asymptomatic carriers of *Campylobacter* 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 (Humphrey *et al.*., 2007). As shown in table (1) results illustrated that liver and gizzard showed the higher incidence of *Campylobacter* contamination than other samples.

It might refer to the original intestinal contamination during bird evisceration (Moore *et al*., 2005). Our result was higher 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; Omara *et al.*, 2015).

Results in table (2) the identification of *Campylobacter* strains isolated from examined samples showed that *C. jejuni, C. coli* and *C.butzieri* were identified in the rate of 28%,20%and 12% in breasts with skin samples respectively, *C. jejuni C. coli and C. lari* were identified in rate of 24% 16% and 8% respectively in breasts without skin. In thighs with skin the rate of isolation of C. *jejuni, C. coli* ,*C. lari* and *C. cinaed were* 40 %16%), 8 % and 8% respectively. In thighs without skin, the rate of isolation of *C. jejuni, C. coli* ,*C.lari* and *C. upsaliens* were 36%, 12% 12% and 8 % respectively Livers were contaminated with *C. jejuni, C. coli C. lari* and *C. cinaediin* rate of 48 %, 20 %, 8%and 4 % respectively . Gizzards were contaminated with *C. jejuni, C. coli* and *C. lari in rate*, of 44 %, 24 % and 12% respectively finally nuggetes were contaminated with *C. jejuni* and *C. in rate*, of 12 %, and 8% respectively. Among the zoonotic Campylobacter species, e.g. *C. jejuni, C. coli, C. lari* and *C. upsaliensis*, the former two species are responsible for the vast majority of the human food borne infections, accounting for 90% and 5-10% of cases (Mikulić *et al.*, 2016).

The zoonotic Campylobacter jejuni is one of the most poultry harboring pathogens, with high public health hazard usually associated with chickens, sequence to the superior levels of human consumption. (Humphrey *et al.*, 2007).

Photo (1) showed the presence of *hipO* gen in isolated c. jejuni. 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).It is the first *Campylobacter* genome to be sequenced was *C. jejuni* by Parkhill *et al.* (2000). Application of molecular tools, such as PCR (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).

Table (3) and photo(1&2) showed the Occurrence of virulence genes of *C. jejuni* strains isolated fromthe examined samples of chicken meat andgiblets ,virulence genes *cdtA, cdtB* and *cdt C , cdtA* and *cdtC* ,*cdtB*and *cdtC* ,*CdtA, CdtB* and *CdtC* were present in 30% , 15%, 25%, 10% ,15 and 5 of examined of strain of *c.jejuni* respectively these genes are involved mainly in adhesion and invasion and they referred to as virulence factors from this point onwards (Chansiripornchai and Sasipreeyajan, 2009).

In conclusion, the highest incidence of *Campylobacter* strains was founded in chicken

liver and gizzard (80%). Using of hipO gene and cytolethal distending toxin (cdt) is very important for accurate identification and characterization of *Campylobacter jejuni* and the virulence genes of *C. jejuni* strains. The current study could be attributed to the fact that most of chickens are sold in pluck-shop markets that devoid hygienic measure leading to increase the contamination of slaughtered chicken carcasses with *Campylobacter*. Therefore, control of Campylobacter infection in poultry production is a major public health strategy for prevention of Campylobacteriosis.

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