Incidence of *Campylobacter* in slaughtered chicken

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Abstract

A total 2565 samples (1890 samples of frozen chicken, 660 samples of freshly slaughter chicken were collected from neck skin, cloacal skin and drip and 15 samples of washing containers) from super market and slaughter poultry house in Egypt and examined for presence of *Campylobacter*. The *Campylobacter* was detected by 16.7 % of all examined samples. Incidence of *Campylobacter* in frozen samples was 7.94 %, 1.59 %, 9.0 % in neck skin, cloacal skin and drip respectively. The results freshly slaughter chicken of revealed 32.73 % incidence of *Campylobacter* in samples. The incidence of *Campylobacter* in the examined washing water were 100%. The cross contamination was occurred during the slaughter processing. And Continues test of poultry carcasses and by-product before packing and distribution is highly recommended with application of good hygienic measure importance to reduce human infection.

Key words: *Campylobacter*, Incidence, frozen chicken, slaughtered chicken.

Introduction

Campylobactriosis is the major important zoonotic gastrointestinal disease around the world most of cases is caused mainly by *C. jejuni*. Poultry play as an important source in transmission of that disease to human ***(Gormley et al, 2008). Kramer et al (2000)*** examined *Campylobacter* isolated from human and poultry samples and found the similarity in its genotypes. Most human disease caused mainly by *Campylobacter jejuni* but the other species may also cause the same human disease ***(CDC, 2013)***. The best suitable temperature for *Campylobacter* species to grow is between 37°C to 42°C and the normal body temperature of bird is (41°C to 42°C) which is the same temperature whish suitable for grow of *Campylobacter* so bird can carry

the campylobacter. *Campylobacter* bacteria cannot tolerate drying and killed by oxygen. It grow only in places with low oxygen about 5%, number of *Campylobacter* in raw meat or poultry samples can decrease by freezing ***(CDC, 2013)***. *Campylobacter* can normally colonized in the intestinal tract of poultry and are considered the most important source of infection ***(Lindblom and Bertil, 1995).*** Because of *Campylobacter* can colonize normally in the intestine so it can directly contaminate the meat product during evisceration inside the slaughter houses and is a major source of transmission of disease to human ***(Misawa et al, 1996 and Rahimi and Tajbakhsh, 2008).*** The most source of human infection is due to eating ready to eat food which may be in contact with the raw poultry meat, or consumption un-prober cooked poultry ***(Lindblom and Bertil, 1995; EFSA 2013).*** Small amount of bird intestinal content during slaughtering may lead to high level of contamination of poultry carcasses with *Campylobacter* which lead to food poisoning problems to human and applying critical control point system is recommended ***(Byrd et al, 1998 and Berrang et al, 2004).*** Freezing may reduce number of *Campylobacter*, so when obtained carcasses contaminated with *Campylobacter* to freezing point for 1-4 weeks the count is between 0.1 and 2.87 $\log_{10}$ CFU/g**.** *Campylobacter* may still be present after 85 weeks of storage at –18°C ***(EFSA, 2004).*** *C. jejuni* can be isolated from refrigerated, frozen, and combined refrigerated and frozen storage poultry samples ***(Bhaduri and Cottrell, 2004).*** Refrigeration and freezing can affect population of *Campylobacter* so the number of *Campylobacter* may be reduced upon freezing and thawing but the organism is still viable for at least one year at –18°C ***(Beuchat, 1986)*** When an infected bird is slaughtered, *Campylobacter* organis ms can be transferred from the intestines to the meat, *Campylobacter* was found on 47% of raw chicken samples bought in grocery stores ***(CDC, 2013)*** *Campylobacter* has been isolated from different places of poultry processing plants and the two *Campylobacter* species was isolated from 32% of chicken product collected from restaurants. ***(Beuchat, 1986).*** **The main objective of this study is to isolate *Campylobacter* from freshly slaughtered and frozen chicken**

Materials and Methods

A total of 2565 samples were collected from different poultry slaughter houses from Giza government and from imported frozen broiler chicken from different supermarkets. Collected samples included skin of neck, skin around the cloaca and drip. These samples were taken from 630 frozen chicken and 220 freshly slaughtered

chicken and 15 samples were collected from water of washing container in slaughter houses.

Isolation and identification was conducted According to **ISO 10272**-1:**2006**.

Isolation

Enrichment protocols

It was based on Bolton broth media where samples were added to × 9 (weight or volume / volume) Bolton broth and incubated for 24 hours at 42°C in microaerophilic atmosphere.

Plating

Direct plating

Aliquot of sample was plated on two agar plats in parallel, one of which was Karmali agar and the second was CCDA agar.

Agar plates were incubated in a microaerophilic atmosphere (CO_2 10%, O_2 5% and N_2 85 %) at 42°C for 24 – 72 hrs.

After enrichment

After selective enrichment a loopeful of the Bolton broth was stroked on two agar plats in parallel, one of which was Karmali agar and the second was CCDA agar.

All types of agar plates were incubated in a microaerophilic atmosphere (CO_2 10%, O_2 5% and N_2 85 %) at 42°C or 24-72 hrs.

Identification

Colonial morphology *Campylobacter jejuni* colonies are convex metallic gray in color on Karmali agar and on CCD agar they are gray in color, moist flat spreading colonies. *Campylobacter coli* on Karmali agar are convex gray colonies and on CCD agar is creamy gray in color, moist and slightly raised.

Cellular morphology one colony was suspended in drop of distal water for bacterial film preparation. The film was stained with Gram stain and examined under ordinary microscope.

Biochemical reaction Oxidase test, catalas test, Na Hippurate hydrolysis test and Nalidixic acid and Cephalothin sensitivity test.

Results

Shape of *Campylobacter* cells under microscope are Gram negative, short curved rods, S shape or gull wings, It oxidase and catalas positive, *C. jejuni* was positive to Na Hippurate hydrolysis while *C. coli* and *C. larides* were negative, *C. jejuni* and *C. coli* were sensitive to Nalidexic acid while *C. larides* was resist to it, all thermophilic *Campylobacter* were sensitive to Cephalothine.

Results showed that 50 out of 630 (7.94 %) neck skin samples were positive for *Campylobacter*, while only 10 out of 630 (1.59 %) sample of cloacal skin were positive for *Campylobacter* High incidence (9%) was from drip samples. 72 out of 220 (32.73%) samples each of neck skin samples, cloacal skin samples, drip samples were positive. More over samples collected from water of washing container from 3 slaughters houses were 100% positive. (Table 1)

The obtained result revealed that 40% of positive samples were *C. jejuni* and 60 % was *C. coli* of neck skin samples, 0% *C. jejuni* and 100% *C. coli* of cloacal skin samples and 42.86%*C. jejuni* & 57.10% *C. coli* in drip samples of frozen chickens. While it was 19.4% *C. jejuni* and 80.56 % *C. coli* in neck skin, cloacal skin and drip of freshly slaughtered chickens. And the percent of isolation of *Campylobacter coli* in the Water from the washing container was 100%. (Table 2, 3)

Table (1) *Incidence of Campylobacter organism in frozen and fresh chicken sample*

Site of samples						
Neck skin		Cloacal skin		Drip		Water from the washing container
Frozen Samples (No. 630)	Fresh Samples (No. 220)	Frozen Samples (No. 630)	Fresh Samples (No. 220)	Frozen Samples (No. 630)	Fresh Samples (No. 220)	(No. 15)
50(7.94%)	72(32.7%)	10(1.59%)	72(32.7%)	70(9%)	72(32.7%)	15(100%)

Table (2) *positive samples and the percent of isolation of Campylobacter jejuni and Campylobacter coli from frozen chicken*

Site of samples					
Neck skin (No. 50)		Cloacal skin (No .10)		Drip (No. 70)	
C. jejuni	*C. coli*	*C. jejuni*	*C. coli*	*C. jejuni*	*C. coli*
20	30	0	10	30	40
40%	60%	0%	100%	42.86%	57.10%

Table (3) positive samples and the percent of isolation of *Campylobacter jejuni* and *Campylobacter coli* from freshly slaughtered chicken

Site of samples							
Neck skin (No. 72)		Cloacal skin (No. 72)		Drip (No. 72)		Water from the washing container (No. 15)	
C.jejuni	*C. coli*	*C. jejuni*	*C. coli*	*C.jejuni*	*C. coli*	*C.jejuni*	*C. coli*
14	58	14	58	14	58	0	15
19.4%	80.6%	19.4%	80.6%	19.4%	80.6%	0 %	100%

Discussion

Campylobacter is one of the food poisoning micro-organism which causes severe cases of gastroenteritis. Poultry is the main vehicle for transmission of *Campylobacter* to human. The organism is commensal microorganism in intestinal tract of birds with or without any clinical signs on birds. At slaughtering of the bird the organism contaminates the water of scalding and washing containers. It was well known that the poultry carcasses was become contaminated with *Campylobacter* bacteria from their intestinal contents during the slaughter process ***(Berndeston et al, 1992).*** The cross-contamination of broiler carcasses by spilled gut contents at slaughter and evisceration presents a potential hygiene problem in poultry abattoirs. This may be particularly significant when *Campylobacter*-free flocks follow colonized flocks through the processing plant ***(Newell et al, 2001).***

(Hafez et al, 2001) Found that in commercial poultry processing there are a several possibilities for cross contamination especially at the scalding stage. Poultry carcasses intended for sale in unfrozen form are scalded at 50.5 ℃ to 57 ℃ to safeguard the appearance of the product, but these temperatures permit pathogenic microorganism such as *Campylobacter* to survive in scald water and cross- contamination of many carcasses are possible.

In this study 1890 samples taken from frozen chicken were examined. *Campylobacter* were detected in 9% of examined samples, this indicates that *Campylobacter* can survive for long period at frozen temperature and it decreases by time. ***(Hefnawy et al, 1989)*** reached to the same conclusion when examined 225 of frozen chicken and isolated the *Campylobacter* with percent 9.78 % .The data currently available on survival of *Campylobacter* do not include heat resistance, although it includes information on survival at chill and freezing temperature, and at temperatures up to 42^{o}C. ***(EFSA, 2004)*** Mentioned that the reduction which obtained by freezing for 1-4 weeks is between 0.1 and 2.87 $\log_{10}$ CFU/g**.** So *Campylobacter* may still be present after 85 weeks of storage at –18°C.

In this study the obtained result showed that percent of isolation of *Campylobacter* bacteria in the fresh slaughter chicken higher than the isolation from the frozen chicken with incidence 32.73% and 9 % respectively. ***Ali (1992)*** reached to the same results of isolation of *Campylobacter* from drip and surface swab of fresh eviscerated whole chicken carcass was higher than which isolated from frozen whole market chicken carcasses with incidence 22% and 14 % respectively.

In addition, the incidence of *Campylobacter* from fresh slaughter chicken carcasses, drip and skin samples were the same as well as

species which isolated from water samples, this indicate that the cross contamination is done through the washing of carcass, this agree with ***Berndeston et al (1992)*** who Reached to the same results that poultry carcasses can become contaminated with *Campylobacter* bacteria from their intestinal contents during the slaughter process.

In these study 15 samples from water of washing, scalding container were examined for isolation of *Campylobacter* bacteria, and found that all samples were positive for isolation of *Campylobacter* bacteria with incidence 100%. ***(Rogol et al, 1984)*** Reached to the same results of isolation *Campylobacter* from all samples collected from washing container by incidence 100 %. However these results is differ from the finding of ***(Hafez et al, 2001)*** when examined 30 samples from scalding water for *Campylobacter* from 6 out 10 monitoring flocks revealed that only one sample was positive for *Campylobacter* bacteria from 30 samples (3.33%).

The rate of isolation in this study of *Campylobacter coli* is higher than the isolation of *Campylobacter jejuni* by incidence in frozen sampled 61.5% and 38.5% respectively. And freshly slaughtered samples, 80.6% and 19.4% respectively.***(Wesley et al, 2005)*** Reached to the same results when examined 5 flocks and found that *Campylobacter coli* was predominant than *Campylobacter jejuni* in the $5^{\underline{th}}$ flock with incidence 82.35% and 17.65% respectively. In contrast ***Nagla Tolba (2005)*** was examined 300 chicken samples and isolated *Campylobacter jejuni* and *Campylobacter coli* by incidence 51.1% and 43.2 % respectively. And ***(Weam Ibrahim, 2005)*** was examined 200 samples of poultry and poultry products and isolated *Campylobacter jejuni* and *Campylobacter coli* by incidence 67.3% and 18.8 % respectively.

Conclusion

The *Campylobacter* is one important food poisoning micro-organism which transmits to human from the consumption of under cooked poultry or misses handling of poultry carcasses so from that indicate the poultry plays a main role in transmission of *Campylobacter* infection to human.

The cross contamination was occurred during poultry processing. The poultry carcasses can become contaminated with *Campylobacter* bacteria from their intestinal contents.

Continuous poultry and poultry by products evaluation and controlling the *Campylobacter* should be carried out through application of good hygienic measures in order to reduce the human infection.

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