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RESEARCH ARTICLE

OCCURRENCE OF CAMPYLOBACTER JEJUNI IN CHICKEN MEAT AND CHICKEN MEAT PRODUCTS

Abd El-Malek, A. M.

Assistant Professor of Meat Hygiene, Department of Food Hygiene, Faculty of Veterinary Medicine, Assiut University, 71515 Assiut, Arab Republic of Egypt

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Corresponding Author

Abd El-Malek, A. M.

Abstract

Background: *Campylobacter jejuni* is a Gram negative, microaerophilic, non-spore-forming, motile and spiral-shaped rod which is able to cause foodborne infection in human called campylobacteriosis. **Aim:** This study was carried out to determine the occurrence of *C. jejuni* in chicken meat and chicken meat products. **Methodology:** A total of 163 samples of chicken meats and chicken meat products including 35 chicken meat samples and 128 chicken meat products were investigated for the presence of *C. jejuni*. To isolate the bacterium, the samples were initially enriched in Bolton broth medium and subsequently transferred to Campylobacter Charcoal Deoxycholate Agar (CCDA). The biochemical tests were used for identification of isolated bacteria at species level. Then these isolates were subjected to further confirmation by Polymerase Chain Reaction (PCR) by targeting *mapA* gene. **Results:** Four fresh chicken breast samples, two fresh chicken thigh and one frozen minced chicken meat were positive for *C. jejuni*. **Conclusion:** In this study, examined fresh chicken breast, fresh chicken thigh and frozen minced chicken meat were found contaminated with *C. jejuni*, so consumption of undercooked or cooked contaminated chicken and chicken products presented a possible risk for consumers. Hence, it is recommended to implement effective hygienic preventive measures to prevent campylobacteriosis in human from chicken meat.

INTRODUCTION

Campylobacter spp. are Gram –ve bacilli, microaerophilic, non-spore-forming, motile and spiral-shaped rod that pose a major public health problem worldwide (Salehi et al., 2014).

The most important pathogenic strains belonging to the group of thermotolerant *Campylobacter* is *C. jejuni*. This species is most often implicated as the causative agent of Campylobacteriosis (Skirrow, 1998).

Campylobacteriosis is a zoonosis, a disease transmitted to humans from animals or animal products. Although in humans such infections are generally self-limiting, complications can arise and may include bacteraemia, Guillain-Barré syndrome, reactive arthritis and abortion (Borsoi et al., 2015).

Studies have identified consumption of contaminated raw or insufficiently cooked chicken products as the major vehicle for campylobacteriosis (Moore et al., 2005 and Zorman et al., 2006).

Meat processing technology has development of variety of convenience and value added products such as chicken nuggets, chicken pane, chicken minced meat, chicken meat balls (kofta), chicken burger, chicken frankfurter and chicken luncheon. Among these, chicken nuggets occupy a predominant place worldwide due to their characteristics flavour and pronounced chewy texture (Muthulakshmi, 2010).

As there are very limited studies done on the prevalence of *C. jejuni* in these kinds of chicken products in Assiut city, Egypt, therefore the present study was conducted to determine the occurrence of *C. jejuni* in chicken meat and chicken meat products retailed in Assiut city.

MATERIALS AND METHODS

Isolation of campylobacter species from chicken meat and chicken by-products:

Samples collection: A total of 163 random samples of chicken meat and chicken by-products were collected from various supermarkets and butcher's shops in Assiut city. The collected samples included fresh chicken meat (15 chicken thigh and 20 chicken breast fillets) and chicken products [19 frozen chicken nuggets (half cooked), 28 frozen crispy chicken pane, 10 frozen chicken minced meat, 12 frozen chicken meat balls (kofta), 15 frozen chicken burger, 18 chicken frankfurter and 26 chicken luncheon]. Chicken meat samples were cut into small pieces by sterile blades for liberation of adherent bacteria to the enrichment broth. This step was done under sterile conditions.

Isolation of *C. jejuni* (Bolton et al., 1984): Ten g of each sample were weighed and placed in sterile bags. Chicken meat samples were homogenized with 90 ml of modified Bolton broth supplemented only with cefoperazone (33 mg per l), amphotericin B (4 mg per l) and 5% lysed horse blood. Samples were enriched for 48 h at 42° C under microaerobic atmosphere (10% CO_2, 5% O_2, and 85% N_2)**.** A loopfull from the incubated broth culture was streaked onto Campylobacter Charcoal Deoxycholate Agar (CCDA, Biolife, Italiana) plates supplemented with 10 mg/l of amphotericin B and 32 mg/l of cefoperazone (Biolife, Italiana) and the plates incubated for 44 ± 4 h at 41.5 ±1 ˚C under an atmosphere of 5% O_2, 10% CO_2 and 85% N_2.

Identification of isolated *C. jejuni*: The isolated colonies that grew on CCDA agar were identified based on morphological appearance and Gram-staining. Biochemical tests were conducted for confirmation of the species using three biochemical tests; namely the urease test, indoxyl acetate test and hippurate hydrolysis test for the presumptive identification of *Campylobacter* spp. (Colles et al., 2003).

DNA Extraction and PCR Conditions*:*

The ingredients necessary for PCR:

1- Template DNA or chromosomal DNA: DNA extracted from fresh cultures of *C. jejuni* according to the manufacturer's instructions of the commercial kit (QIAamp DNA Mini & Blood Mini Kit, Cat.no. 51104).

2-Single stranded oligonucleotide primers: Two oligonucleotide primers were designed and synthesized according to the determined sequence of *mapA* gene from *C. jejuni* **(**Denis et al., 1999**).**

Table 1. Specific primer sequences for the detection of *C. jejuni*

Primer name (pmol/µL)	Primer sequences	Target gene	Amplicon length specificity	Reference
Primer 1:MDmapA1 (10): Primer 2: MDmapA2 (10):	**5` - CTA TTT TAT TTT TGA GTG CTT GTG - 3`** (Forward) **5` - GCT TTA TTT GCC ATT TGT TTT ATT A - 3`** (Reverse)	*mapA*	589 bp	Denis et al., 1999

3- PCR beads (Ready- to- Go™ PCR Beads) (Amersham Pharmacia Biotech, Austria): PCR beads designed as pre-mixed predispensed reactions for performing PCR amplifications. Each bead contains all of the necessary reagents, except primer and template, for performing individual PCR reaction.

PCR Procedures: PCR amplification was performed in a total volume of 50 µl containing 25 µl [Enzyme (DNA polymerase, *Taq)*, dNTPs (A, T, G, C), Buffer (50 mM KCl; 10 mM Tris-HCl; 1.5 mM MgCl2], 30 - 50 ng of DNA extracts and 20 pmol of each primer. Amplification reactions were carried out using thermal cycler (Thermacycler Biometra TProfessional) with the following program: an initial denaturation at 95°C for 3 min followed by 45 cycles of denaturation at 94°C for 40 sec, annealing at 41°C for 40 sec and polymerization at 72°C for 1 min. A final extension was performed at 72°C for 10 min. The amplification generated 589bp DNA fragments corresponding to *C. jejuni*. The PCR products were stained with a 0.5% solution of ethidium bromide and were visualized under UV light after gel electrophoresis on 1% agarose and a molecular weight marker ladder (Amersham) was included.

RESULTS AND DISCUSSION

Table 2. Prevalence of *C. jejuni* in examined fresh chicken and frozen chicken products

Products	No. of examined samples	Campylobacter spp.		*C. jejuni*	
		+ve	%	+ve	%
Fresh chicken breast	20	6	30	4	20
Fresh chicken thigh	15	2	13.33	2	13.33
Frozen chicken nuggets	19	0	0.0	0	0.0
frozen chicken pane	28	0	0.0	0	0.0
frozen chicken kofta	12	0	0.0	0	0.0
Frozen minced meat	10	2	20	1	10
frozen chicken burger	15	0	0.0	0	0.0
chicken frankfurter	18	0	0.0	0	0.0
chicken luncheon	26	0	0.0	0	0.0

Figure 1. *C. jejuni* specific Polymerase Chain Reaction

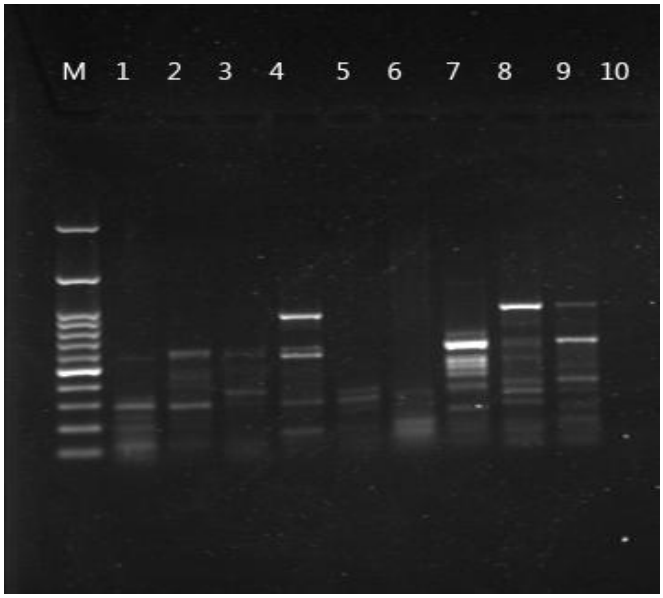

Fig 1. PCR amplicon (589-bp) using *mapA* gene in fresh chicken and chicken product samples. M: 100 base pair ladder marker; Lane 1-4 and 7-9: PCR Positive band for *C. jejuni*; Lane 5-6 and 10: Negative for *C. jejuni*.

Our findings in Figure (1) illustrated electrophoretic analysis of 1 % agarose gel stained by Ethidium bromide of PCR amplification products of our tested cases confirmed the diagnosis of *C. jejuni* isolated from fresh chicken meat and frozen minced chicken meat samples. The amplified product of this positive sample corresponded to 589 bp M.W.

Data outlined in Table (2) showed that *C. jejuni* can be found in 4 of the examined fresh chicken breast fillet samples with an incidence of 20%. In contrast, lower results (6.9%) concerning *C. jejuni* obtained in a related study in Zagazig city, Egypt conducted by Awadallah et al. (2014), while higher records 66% identified by Williams and Oyarzabal (2012) in Alabama, USA.

On the other hand, the achieved data in the present study disagree with Kozačinski et al. (2006) who reported that Campylobacter spp. were not found in any of analysed samples of fresh chicken breast.

The processing of boneless, skinless chicken breasts entails greater handling by workers, as it is a further processed food. So, the potential for contamination with *Campylobacter* spp. is high (Rasmussen, 1999).

The findings presented in table (2) revealed that *C. jejuni* were isolated from 2 out of 15 examined fresh chicken thigh samples with an incidence of 13.33%. On the other hand, lower incidence (6.9%) was isolated from chicken thigh meat samples in Zagazig city, Egypt (Awadallah et al., 2014). Meanwhile, higher percentage (70%) obtained in Alabama, USA by Williams and Oyarzabal (2012).

Campylobacters may colonize the intestines of clinically healthy birds, and therefore the poultry products and chicken meat are considered to be the main source of foodborne Campylobacter infection (EFSA, 2013).

Therefore, consumption of undercooked chicken meats may cause food poisoning and complete cooking of poultry is critical to prevent this infection (Rahimifard et al., 2009).

Aho and Hirn (1988) recorded the prevalence of Campylobacters in several developed and developing countries which ranged from 6% in Sweden to 100% in Italy. Other studies also showed that *C. jejuni* is more commonly found in chickens (Humprey et al., 1993; Aho and Hirn, 1988) compared to other Campylobacter spp.

The results demonstrated in Table (2) revealed that microbiological analysis done on marinated frozen chicken-based products (chicken nuggets, crispy chicken pane and chicken kofta) showed the absence of *C. jejuni.* These obtained results of this study were in agreement with other related studies and reports as NurIlida and Faridah (2012).

A marinade is a liquid mixture in chicken is soaked prior to cooking and contains spices, herbs, salts, oil and an acid (Faridah, 2002). In marinated chicken (frozen chicken nuggets (half cooked), frozen crispy chicken pane and frozen chicken meat balls (kofta), failure of isolating *C. jejuni* could be due to a high concentration of salt and other ingredients contained in the marinades. The *Campylobacter* can usually survive up to 2.0% of salt (Rob et al., 2003). So in this case, it was possible that the samples might have been contaminated by *C. jejuni*, but later was killed by the use of marinades.

There are very limited studies done on the prevalence of Campylobacter in these kinds of frozen chicken products worldwide (Ilida and Faridah, 2012). Even though frozen chicken products may be at a lower risk in supporting the growth of *Campylobacter*, there are possibilities of isolating *C. jejuni* from contaminated frozen products (Rob et al., 2003). This is because the numbers of *C. jejuni* decline slowly at normal freezing temperatures and therefore does not instantly activate the organism in foods.

The obtained results showed in Table (2) showed that frozen minced chicken meat were contaminated with *C. jejuni* with percentage 10%. On the other hand, Kozačinski et al. (2006) failed to isolate *C. jejuni* from frozen ground chicken meat. On contrary, higher incidence (31.3%) of *C. jejuni* were isolated from minced chicken meat samples in Japan (Fukushima et al., 2007).

In general, the comparison between different studies should be prudent as reported variations in Campylobacter spp. prevalence may be due to the use of different sampling and analytical techniques employed, as well as to season-related differences (Rahimi & Tajbakhsh, 2008).

The PCR assay offers an alternative to traditional biochemical typing methods for the identification and differentiation of *C. jejuni* isolated from poultry. It is accurate, simple to perform, and can be completed within 8 h (Harmon et al., 1997).

CONCLUSION

In this study, examined fresh chicken breast, fresh chicken thigh and frozen minced chicken meat were found contaminated with *C. jejuni*, so consumption of undercooked or cooked contaminated chicken and chicken products presented a possible risk for consumers. Hence, it is recommended to implement effective hygienic preventive measures to prevent campylobacteriosis in human from chicken meat.

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