World Journal of Dairy & Food Sciences 7 (2): 222-228, 2012
ISSN 1817-308X

DOI: 10.5829/idosi.wjdfs.2012.7.2.56167

Occurrence and Enumeration of Campylobacters Before and After Processing of Conventional Chickens in Egypt

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Abstract: *Campylobacter* is considered to be the most common cause of bacterial diarrheal illness and many cases are thought to be acquired from consumption of undercooked poultry or through cross-contamination of other foods during the preparation of poultry. The numbers of cases of human disease in Egypt with *Campylobacter* have increased but nothing is known about the specific source of infection in Egypt or about the incidence of *Campylobacter* in Egyptian reared poultry. The aim of this study was therefore to enumerate both campylobacters and total bacterial counts in four conventional chicken Egyptian flocks in North Sinai governorate, starting from the farm and progressing to the slaughterhouse. Samples were taken from chicken carcasses at six different stages during processing. The effect of post-slaughter chilling on the survival of campylobacters at 4°C and -20°C was also compared. All four flocks were infected with *Campylobacter* spp. before processing and these were also detected at the slaughterhouse on the chicken carcasses where *C. jejuni* was the dominant species. The highest *Campylobacter* counts, (mean 9.3 $CFUg^{-1}$), were detected in the intestinal contents of live birds, but high levels of contamination were also found on fresh cooled and frozen chicken meat samples (7.4 $CFUg^{-1}$ and 7.8 $CFUg^{-1}$ respectively). This high level and the continuous presence of *Campylobacter* in the slaughterhouse constitute a risk for transmission to negative carcasses. Our findings indicate that chilling and freezing processes have a limited effect in reducing contamination of final products. As in other parts of the world, chickens in Egypt that enter the slaughterhouse contaminated with *Campylobacter* represent a risk to consumers' health in cases of improper handling or cooking practices.

Key words: *Campylobacter* · Occurrence · Enumeration · Survival · Chicken · Processing

INTRODUCTION

Campylobacters are Gram-negative bacteria that are ubiquitous in the environment [1] and considered as a bacterial cause of enteric disease [2]. In developing countries *Campylobacter jejuni* enteritis usually occurs in infants whereas it is rare in adults [3]. *Campylobacter* isolation rates in developing countries range from 5 to 20% [4, 5]. In 2000, Wasfy *et al*. [6] isolated 146 campylobacters from the stools of 6278 Egyptian patients. In another study, Rao *et al*. [7] reported that most of Egyptian children aged less than 3 years are infected with *Campylobacter* at 0.6 episodes per child a year and they attributed this high rate to the presence of animals and birds in the house in addition to the poor hygienic practices. About 100 of 118 *Campylobacter* isolates from Egyptians during 1998-2005 were found to be resistant to fluoroquinolones [8].

Chickens are considered major reservoirs of *Campylobacter* infection [9] and significant relationship has been reported between sporadic human *Campylobacter* infection and the handling and preparation of chickens or consumption of undercooked chickens [10-11]. About 40% of tested chicken lever samples in Egypt were positive for *Campylobacter* [12]. In a recent study, *Campylobacter* was isolated from 39.17% of broiler carcass samples in Assiut city in Egypt [13]. Human can be infected with this bacterium with a relatively low dose (500 CFU) through cross-contamination of other food products or direct hand to mouth contact after handling raw chicken [14, 16].

Campylobacters are usually undetectable in intensively reared chickens until at least 10 days of age and most of chickens become colonized with campylobacters after 2 to 3 weeks [17]. This may be due to the protective activity of maternal antibodies against

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Campylobacter colonization after hatching [18, 19]. *Campylobacter* appears to be so well adapted to the poultry gut that as few as 100 organisms can colonize [20]. Gastrointestinal tract; in particular the ceca, small intestine, large intestine and cloaca is the principle site of colonization with this microorganism [21]. Approximately three out of four live chickens and more than 80% of retail poultry meat are contaminated with *Campylobacter* [22, 23]. In one study of raw chicken meat sold in the UK, 80% was contaminated with *Campylobacter* [24] with 98 and 2% of isolates identified as *C. jejuni* and *C. coli,* respectively. *C. jejuni* is considered as the most important cause for foodborne gastroenteritis worldwide [25]. Therefore, large numbers of campylobacters can be dispersed during transport and abattoir processing causing a possible hygiene problem in abattoirs.

Campylobacter-positive chickens entering the slaughter line can cause extensive cross-contamination to non infected carcasses during slaughtering and processing and even after chilling and cutting of poultry products and represents a significant challenge for the poultry industry [26]. The surface of the chickens can be contaminated with this microorganism after scalding and defeathering and these numbers are reported to be in the region of 5.9 log CFU per carcass [27]. Most of chicken flocks become contaminated by *Campylobacter* spp. on the day of slaughter. It has been reported that refrigeration of poultry meat is very useful to control the growth of pathogenic organisms such as *Campylobacter* [28]. Previous studies have shown that freezing to -20°C and cooling to 4°C reduce viable counts of campylobacters by causing lethal or sub lethal injuries [29, 30]. However, this will not significantly reduce the numbers of campylobacters associated with the chicken skin [30].

In this study we described the enumeration of both *Campylobacter* and total bacteria isolated during the rearing cycle of conventional chickens and also during processing. We also studied the effect of cooling and freezing in a domestic refrigerator on the survival characteristics of *Campylobacter*.

MATERIALS AND METHODS

Source of Samples: Four conventional chicken farms were randomly selected at different geographic locations in El-Arish city, North Sinai governorate. All birds in this study were Ross broilers from a commercial hatchery. Birds were reared on single species farms with up to 5.000 birds kept in a single shed. Soil and fresh fecal samples were collected from the farm at the end of the trial. At the slaughterhouse, samples from specimens during slaughtering were obtained using the standard method. The slaughterhouse was divided into 7 areas according to the ongoing activity: arrival, scalding, defeathering, evisceration, final wash, refrigeration and freezing stages. Three birds from each flock were investigated at the different processing stages from arrival and onwards. For the defeathering, final wash, refrigeration and freezing stages, an area of at least 100 cm^2 of the surface of each of 5 carcasses per stage was cut, aseptically transferred to a plastic bag. In addition, at evisceration, 10 whole ceca were collected from each flock under study, transferred to sterile plastic bag to the laboratory.

Isolation and Enumeration of *Campylobacter* from Conventional Chickens: Three birds were selected at random from the conventional flocks during abattoir processing. Fresh soil and excreta samples were collected from chickens before slaughtering. Birds were removed from the flock and transported directly to the abattoir for slaughtering and for commercial processing. After slaughtering, the ceca from sample birds were removed by sterile dissection and the contents were collected for *Campylobacter* and total bacterial isolation. During abattoir processing chicken samples were removed after defeathering, eviscerating, after final wash and also after refrigeration (after 3 days of storage) and freezing (after 20 days of storage) for *Campylobacter* isolation. All samples were transported to the Food Microbiology Laboratory (Faculty of Environmental Agricultural Sciences, Suez Canal University, Egypt) under refrigerated conditions. Serial dilutions were made by using the Maximum Recovery diluent as described before [31] (catalogue no. CM733; Oxoid, Basingstoke, United Kingdom). Volumes (100 µl) of each diluted sample were then spread on the surface of the modified cefoperazone charcoal deoxycholate agar (mCCDA) selective medium (CM739 and selective supplement SR155; Oxoid). The plates were then incubated at 42°C for 48 h under microaerobic conditions (5% O_2, 5% H_2, 10% CO_2 and 80% N_2). Ten or more typical *Campylobacter* colonies were examined by Gram stain and wet mount and sub cultured on blood agar [blood agar base number 2 (CM 271; Oxoid) with horse blood]. Oxidase and catalase tests were performed and the identification of the isolates was

confirmed by using the hippurate test. A loopful of a fresh *Campylobacter* culture was emulsified in 500 µl of 5% (w/v) hippuric acid (Sigma: H-9380) and incubated aerobically at 37°C for 2 h. After incubation, the suspension was overlaid with 200 µl of 3.5% (w/v) ninhydrin solution (BioMerieux: REF 70 490) for a further 10 minutes. A positive result is indicated by the development of a deep blue colour whereas a colourless or light to medium purple coloration is considered negative [32]. Some of the isolates were identified by species specific PCR with primers specific to *hip*O and putative aspartokinase gene and flanking ORF target genes described by Linton *et al*. [33]. *Campylobacter* DNA for PCR analysis was extracted using the protocol determined by Pitcher *et al*. [34].

Enumeration of Total Bacteria Isolated from Conventional Chickens: Samples were diluted by using Maximum Recovery diluent and volumes (100 µl) of each dilution were then spread on the surface of plate count agar (Oxoid, CM0463) and incubated at 37°C for 24 h under aerobic conditions [31].

Enumeration of Campylobacters and Total Bacteria Isolated from Chickens` Skin: The samples were aseptically transferred into sterile plastic bags containing 10 ml of Maximum Recovery diluent and mixed appropriately for 5 min. The suspension was serially diluted ten folds, spread and incubated as described before [31].

Statistical Analysis: Statistical analysis was performed on $\log_{10}$ transformed values using the means of triplicate viable count values, for each data point obtained. The data were analyzed using the Microsoft Excel software package (Microsoft Corporation, Redmond, Washington, USA).

RESULTS

In this study, quantitative examination for both total bacteria and *Campylobacter* in the investigated samples (total 188) before and after processing of the conventional chickens (Table 1) was performed. The present study showed high prevalence of *Campylobacter* in chicken samples during slaughtering process. Overall, *Campylobacter* contamination, ranged from 7.4 $\log_{10}$ up to 9.3 $\log_{10}$ $CFUg^{-1}$. Both soil and fecal samples were highly contaminated with *Campylobacter* and the observed mean counts were 8.3 $\log_{10}$ and 8.6 $\log_{10}$ $CFUg^{-1}$ respectively. The isolation rate at the pre-evisceration stage was quite high (mean counts 8.0 $\log_{10}$ $CFUg^{-1}$) due to feathers and skin contamination with feces from transportation through the defeathering stage.

As expected, the highest *Campylobacter* counts mean (9.3 $CFUg^{-1}$) was in intestinal contents. Also, the carcasses were contaminated with higher numbers of *Campylobacter* after evisceration (8.4 $CFUg^{-1}$). The mean count of *Campylobacter* after final washing was quite similar to the mean count after evisceration (8.4 $CFUg^{-1}$). The *Campylobacter* mean values detected in cooled and frozen carcasses were significantly lower ($P < 0.05$) than those detected in intestinal contents (Table 1). All the 40 fresh cooled and frozen chicken meat samples examined were *Campylobacter* positive and the observed counts were (7.4 $CFUg^{-1}$) and (7.8 $CFUg^{-1}$) respectively.

The results of this study showed that the thermotolerant *Campylobacter* contamination was prevalent in caecal ontents and the poultry carcasses and that all four broiler flocks were colonized with *Campylobacter* (100%) (Illustrated in Table 1). Both conventional hippurate test and polymerase chain reaction (PCR) differentiation of the *Campylobacter* isolates revealed that *C. jejuni* was the dominant species among isolates from all flocks before and after processing of the conventional chickens (Fig. 1).

Table 1: Quantification of total bacteria and *Campylobacter* before and after processing

Sample	Number of samples tested (n)	Total bacterial counts mean log $CFUg^{-1}$(SD)	*Campylobacter* counts mean log $CFUg^{-1}$ (SD)
Soil	24	7.9 (± 0.07)	8.3 (± 0.06)
Faecal	24	9.3 (± 0.20)	8.6 (± 0.19)
Intestine	40	9.9 (± 1.74)	9.3 (± 1.78)
After defeathering	20	8.7 (± 1.69)	8.0 (± 2.44)
After evisceration	20	9.4 (± 1.47)	8.4 (± 2.04)
After final wash	20	9.1 (± 1.93)	8.4 (± 1.57)
After refrigeration	20	8.1 (± 1.63)	7.4 (± 2.17)
After freezing	20	7.3 (± 1.46)	7.8 (± 2.05)
Total	188		

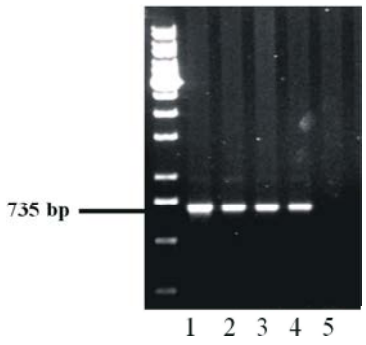

Fig. 1: Agarose gel showing PCR products obtained from genomic DNAs of conventional chicken isolates using a primer specific to *hip*O target gene. A) Lane 1 DNA marker 1 kbp; Lanes 2 to 5 *C. jejuni* isolate DNA that amplified with *hip*O gene primers

DISCUSSION

Campylobacteriosis is considered as a serious foodborne disease worldwide [35] and poultry meat contaminated from intestinal contents of colonized chickens is the major source of infection with *Campylobacter*. Most of Egyptians like poultry meat and they enjoyed chicken the most. The commercial poultry sector in Egypt became more dynamic. Most of Egyptians prefer live birds which can be slaughtered immediately at sale to consumers and it constitute about 70% of chicken production and the remaining 30 percent is sold frozen [36]. The Egyptian chicken sector is subdivided into commercial (international breeds) and Balady (traditional breeds). The fate of *Campylobacter* populations following chicken slaughter in Egypt is almost unknown. Handling of raw chicken meat at home bears the risk of cross-contamination, particularly in the case of unsatisfactory kitchen hygiene [14]. However, campylobacters are almost susceptible to environmental stresses such as oxygen and heat and as a result their viable numbers decline in the environment.

The results of this study showed that the thermotolerant *Campylobacter* contamination was prevalent in caecal contents and the poultry carcasses and that all four broiler flocks were colonized with *Campylobacter* (100%) and this could be regarded as high, compared with other countries with proportions varying from about 10 to 90% [1]. This result is not surprising because cross contamination with *Campylobacter* in the farm and during processing is very common and unavoidable. Our study showed that *C. jejuni* was the dominant species and this result is in agreement with previous studies with intensively reared birds which stated that 80 to 90% of isolates were *C. jejuni* and the remainder was *C. coli* [24, 38].

Since slaughtering process involved many steps *Campylobacter* cross contamination is very likely to occur. The contamination of carcasses with *Campylobacter* increased during evisceration steps. The evisceration process contributes significantly to *Campylobacter* contamination [39] and this increase in contaminated carcasses is due to the rupture of intestine which leads to the contamination of equipment and working surfaces and increase the opportunities for cross contamination of *Campylobacter*-free carcasses during processing [22, 40]. Once the surface of a carcass is contaminated with *Campylobacter*, such contamination has the potential to persist through the rest of the carcass processing [41, 42]. Accordingly, special attention should be given to control the potential contamination with *Campylobacter* during processing, such as the plucking and evisceration steps so as to reduce cross contamination with *Campylobacter* during following processes.

Good practices during poultry processing such as decontamination of equipment, the use of counter flow multistage water systems during scalding and disinfecting the carcasses might reduce the poultry contamination with campylobacters [22]. The changes in bacterial viability during processing of poultry carcasses are due to different stresses including nutrient deprivation and changes in temperature, oxygen tension and the osmolarity [37]. Insufficient cleaning and disinfection will support the survival of *Campylobacter*, up to 1 week, which is most often found in humid and wet places in the slaughterhouse [26]. This survival in the environment through washing and disinfection may be due to *Campylobacter* remaining in biofilm layers [43].

Since chicken meat is a perishable food it must be stored either refrigerated or frozen throughout the food chain in order to avoid and reduce microbial contamination and growth. Previous studies showed that *Campylobacter* can survive during refrigerated (4°C) and frozen (-20°C) storage on various chicken meat [30, 44]. The results of this study showed that cooled and frozen chicken meat samples from the slaughterhouse were positive for *Campylobacter* and the numbers of *Campylobacter* were decreased to 7.4 and 7.8 $\log_{10}$ compared to the numbers of *Campylobacter* after evisceration (8.4 $CFUg^{-1}$). On the other hand,

El-Shibiny *et al*. [30] and Sampers *et al*. [45] stated that 0.9 to 3.2 $\log_{10}$ reductions were observed in viable *Campylobacter* counts on chicken skin after storage at refrigeration and frozen temperature. Contamination of carcasses at the slaughterhouse may be due to the leakage of the gastrointestinal tract after its rupture which has high numbers of *Campylobacter*, with levels up to 10^9 CFU/g of intestinal content. Consequently, cross-contamination during processing at the slaughterhouse is considered the main risk factors for the presence of *Campylobacter* spp. on poultry carcasses [46, 47]. Seeing that *Campylobacter* is able to grow between 30 and 46°C and require micro aerobic conditions, it is unlikely that numbers of the organism will increase on contaminated carcasses during storage. However, *Campylobacter* might persist in refrigerated and frozen stored chicken meat long enough to recover then pose a health risk to humans [30].

In conclusion, reducing contamination on the farm and monitoring *Campylobacter* contamination at different stages of chicken production are more likely to be effective than chilling or freezing.

ACKNOWLEDGMENT

This work was supported by Food and Dairy Sciences and Technology department, Faculty of Environmental Agricultural Sciences, Suez Canal University, El-Arish, North Sinai. The authors would like to thank all chicken producers and slaughterhouses owners for their helping to achieve this work.

REFERENCES

1. Newell, D.G. and C. Fearnley, 2003. Sources of *Campylobacter* colonization in broiler chickens. Appl. Environ. Microbiol., 69: 4343-4351.
2. Adak, G.K., S.M. Long and S.J. O'Brien, 2000. Trends in indigenous foodborne disease and deaths, England and Wales: 1992 to 2000. Gut, 51: 832-841.
3. Ketley, J.M., 1997. Pathogenesis of enteric infection by *Campylobacter*. Microbiol., 143: 5-21.
4. Oberhelman, R.A. and D.N. Taylor, 2000. *Campylobacter* infection in developing countries. In *Campylobacter*, Eds., I. Nachamkin and M.J. Blaser. American Society for Microbiology, Washington D.C., pp: 139-154.
5. Coker, A.O., R.D. Isokpehi, B.N. Thomas, K.O. Amisu and C.L. Obi, 2002. Human campylobacteriosis in developing countries. Emerg. Infect. Dis., 8: 237-244.
6. Wasfy, M.O., B.A. Oyofo, J.C. David, T.F. Ismail, A.M. El-Gendy, Z.S. Mohran, Y. Sultan and L.F. Peruski, 2000. Isolation and antibiotic susceptibility of *Salmonella*, *Shigella* and *Campylobacter* from acute enteric infections in Egypt. J. Health Popul. Nutr., 18: 33-38.
7. Rao, M.R., A.B. Naficy, S.J. Savarino, R. Abu-Elyazeed, T.F. Wierzba, L.F. Peruski, I. Abdel-Messih and I. Frenck, J.D. Clemens, 2001. Pathogenicity and convalescent excretion of *Campylobacter* in rural Egyptian Children. Am. J. Epidemiol., 154: 166-173.
8. Said, M.M., H. El-Mohamady, F.M. El-Beih, D.M. Rockabrand, T.F. Ismail, M.R. Monteville, S.F. Ahmed, J.D. Klena and M.S. Salma, 2010. Detection of *gyrA* mutation among clinical isolates of *Campylobacter jejuni* isolated in Egypt by MAMA-PCR. J. Infect. Dev. Ctries., 4: 546-554.
9. Shane, S.M., 2000. *Campylobacter* infection of commercial poultry. Rev. Sci. Tech., 19: 376-395.
10. Park, R.W., P.L. Griffiths and G.S. Moreno, 1991. Sources and survival of campylobacters: relevance to enteritis and the food industry. Soc. Appl. Bacteriol. Symp. Ser., 20: 97S-106S.
11. Kapperud, G., G. Espeland, E. Wahl, A. Walde, H. Herikstad, S. Gustavsen, I. Tveit, O. Natås, L. Bevanger and A. Digranes, 2003. Factors associated with increased and decreased risk of *Campylobacter* infection: a prospective case-control study in Norway. Am. J. Epidemiol., 158: 234-242.
12. Khalafalla, F.A., 1990. *Campylobacter jejuni* in poultry giblets. Zentralbl. Veterinarmed. B., 37: 31-34.
13. Sayed, A.S. and A.A. Mohamed, 2004. Surveillance of enteropathogenic *Campylobacter* in raw poultry meat and some poultry products in Assiut City. Assiut Vet. Med., 50: 102.
14. Humphrey, T.J., K.W. Martin, J. Slader and K. Durham, 2001. *Campylobacter* spp. in the kitchen: spread and persistence. Symp. Ser. Soc. Appl. Microbiol., pp: 115S-120S.
15. Friedman, C.R., J. Neimann, H.C. Wegener and R.V. Tauxe, 2000. Epidemiology of *Campylobacter jejuni* infection in the United States and other industrialized nations. In: *Campylobacter*, Eds., Nachamkin, I. and M.J. Blaser. American Society for Microbiology, Washington D.C., pp: 121-138.
16. Jacobs-Reitisma, W., U. Lyhs and J. Wagenaar, 2008. *Campylobacter* in the food supply. In: *Campylobacter,* Eds., Nachamkin, I., C.M. Szymanski and M.J. Blaser., 3rd Edition, ASM Press, New York, pp: 627-644.

17. Evans, S.J. and A.R. Sayers, 2000. A longitudinal study of *Campylobacter* infection of broiler flocks in Great Britain. Prev.Vet. Med., 46: 209-223.
18. Cawthraw, S., R. Ayling, P. Nuijten, T. Wassenaar and D.G. Newell, 1994. Isotype, specificity and kinetics of systemic and mucosal antibodies to *Campylobacter jejuni* antigens, including flagellin, during experimental oral infections of chickens. Avian Dis., 38: 341-349.
19. Sahin, O., N. Luo, S. Huang and Q. Zhang, 2003. Effect of *Campylobacter*-specific maternal antibodies on *Campylobacter jejuni* colonization in young chickens. Appl. Environ. Microbiol., 69: 5372-5379.
20. Young, C.R., R.L. Ziprin, M.E. Hume and L.H. Stanker, 1999. Dose response and organ invasion of day-of-hatch Leghorn chicks by different isolates of *Campylobacter jejuni*. Avian Dis., 43: 763-767.
21. Beery, J.T., M.B. Hugdahl and M.P. Doyle, 1988. Colonization of gastrointestinal tracts of chicks by *Campylobacter jejuni*. Appl. Environ. Microbiol., 54: 2365-2370.
22. Jacobs-Reitsma, W., 2000. *Campylobacter* in the food supply. In: *Campylobacter*, Eds., I. Nachamkin and M.J. Blaser, 2nd Edition, ASM Press, Washington, D.C., pp: 467-481.
23. Hiett, K.L., N.A. Cox and N.J. Stern, 2002. Direct polymerase chain reaction detection of *Campylobacter* spp. in poultry hatchery samples. Avian Dis., 46: 219-223.
24. Jorgensen, F., R. Bailey, S. Williams, P. Henderson, D.R. Wareing, F.J. Bolton, A. Forst, L. Ward and T.J. Humphrey, 2002. Prevalence and numbers of *Salmonella* and *Campylobacter* spp. on raw, whole chickens in relation to sampling methods. Int. J. Food Microbiol., 76: 151-164.
25. Reid, A.N., R. Pandey, K. Palyada, H. Naikare and A. Stintzi, 2008. Identification of *Campylobacter jejuni* genes involved in the response to acidic pH and stomach transit. Appl. Environ. Microbiol., 74: 1583-1597.
26. Corry, J.E. and H.I. Atabay, 2001. Poultry as a source of *Campylobacter* and related organisms. Symp. Ser. Soc. Appl. Microbiol., pp: 96S-114S.
27. Reich, F., V. Atanassova, E. Haunhorst and G. Klein, 2008. The effects of *Campylobacter* numbers in caeca on the contamination of broiler carcasses with *Campylobacter*. Int. J. Food Microbiol., 127: 116-120.
28. Murphy, C., C. Carroll and K.N. Jordan, 2006. Environmental survival mechanisms of the foodborne pathogen *Campylobacter jejuni*. J. Appl. Microbiol., 100: 623-632.
29. Lee, A., S.C. Smith and P.J. Coloe, 1998. Survival and growth of *Campylobacter jejuni* after artificial inoculation onto chicken skin as a function of temperature and packaging conditions. J. Food Prot., 61: 1609-1614.
30. El-Shibiny, A., P. Connerton and I. Connerton, 2009. Survival at refrigeration and freezing temperatures of *Campylobacter coli* and *Campylobacter jejuni* on chicken skin applied as axenic and mixed inoculums. Int. J. Food Microbiol., 131: 197-202.
31. El-Shibiny, A., P.L. Connerton and I.F. Connerton, 2005. Enumeration and diversity of campylobacters and bacteriophages isolated during the rearing cycles of free-range and organic chickens. Appl. Environ. Microbiol., 71: 1259-1266.
32. Stern, N.J., C.M. Patton and M.P. Doyle, 1992. In: Compendium for the microbiological examination of foods, Eds., C. Vanderzant, American Public Health Association, Washington D.C., pp: 475-495.
33. Linton, D., A.J. Lawson, R.J. Owen and J. Stanley, 1997. PCR detection, identification to species level and fingerprinting of *Campylobacter jejuni* and *Campylobacter coli* direct from diarrheic samples. J. Clin. Microbiol., 35: 2568-2572.
34. Pitcher, D.G., N.A. Saunders and R.J. Owen, 1989. Rapid extraction of bacterial genomic DNA with guanidium thiocyanate. Lett. Appl. Microbiol., 8: 151-156.
35. Tauxe, R.V., 1992. Epidemiology of *Campylobacter jejuni* infections in the United States and other industrialized nations,. In: *Campylobacter jejuni*: *current status and future trends*. American Society for Microbiology, Eds., I. Nachamkin, M.J. Blaser and L.S. Tompkins, Washington, D.C., pp: 9-19.
36. U.S. Department of Agriculture, Foreign Agricultural Service, 2001. Egypt Poultry and Products, Annual GAIN Report EG1020, Washington, D.C.
37. Newell, D.G., J.E. Shreeve, M. Toszeghy, G. Domingue, S. Bull, T. Humphrey and G. Mead, 2001. Changes in the carriage of *Campylobacter* strains by poultry carcasses during processing in abattoirs. Appl. Environ. Microbiol., 67: 2636-2640.
38. Uyttendaele, M., R. Schukkink, B. Van Gemen and X. Debevere, 1996. Comparison of the nucleic acid amplification system of NASBA and agar isolation for detection of pathogenic *Campylobacter* in naturally contaminated poultry products. J. Food Prot., 59: 683-687.

39. Rosenquist, H., H.M. Sommer, N.L. Nielsen and B.B. Christensen, 2006. The effect of slaughter operations on the contamination of chicken carcasses with thermotolerant *Campylobacter*. Int. J. Food Microbiol., 108: 226-232.
40. Figueroa, G., M. Troncoso, C. Lopez, P. Rivas and M. Toro, 2009. Occurrence and enumeration of *Campylobacter* spp. during the processing of Chilean broilers. BMC. Microbiol., 9: 94.
41. Byrd, J.A., B.M. Hargis, D.E. Corrier, R.L. Brewer, D.J. Caldwell, R.H. Bailey, J.L. McReynolds, K.L. Herron and L.H. Stanker, 2002. Fluorescent marker for the detection of crop and upper gastrointestinal leakage in poultry processing plants. Poult. Sci., 8: 70-74.
42. Saengthongpinit, C., S. Kanarat, T. Sirinarumitr, P. Amavisit and T. Sakpuaram, 2010. Amplified Fragment Length Polymorphism Analysis of *Campylobacter jejuni* and *Campylobacter coli* from Broiler Farms and Different Processing Stages in Poultry Slaughterhouses in the Central Region of Thailand. Kasetsart J. Nat. Sci., 44: 401-410.
43. Trachoo, N., J.F. Frank and N.J. Stern, 2002. Survival of *Campylobacter jejuni* in biofilms isolated from chicken houses. J. Food Prot., 65: 1110-1116.
44. Bhaduri, S. and B. Cottrell, 2004. Survival of cold-stressed *Campylobacter jejuni* on ground chicken and chicken skin during frozen storage. Appl. Environ. Microbiol., 70: 7103-7109.
45. Sampers, I., I. Habib, Z.L. De, A. Dumoulin and M. Uyttendaele, 2010. Survival of *Campylobacter* spp. in poultry meat preparations subjected to freezing, refrigeration, minor salt concentration and heat treatment. Int. J. Food Microbiol., 137: 147-153.
46. Shanker, S., J.A. Rosenfield, G.R. Davey and T.C. Sorrell, 1982. *Campylobacter jejuni*: incidence in processed broilers and biotype distribution in human and broiler isolates. Appl. Environ. Microbiol., 43: 1219-1220.
47. Rivoal, K., M. Denis, G. Salvat, P. Colin and G. Ermel, 1999. Molecular characterization of the diversity of *Campylobacter* spp. isolates collected from a poultry slaughterhouse: analysis of cross-contamination. Lett. Appl. Microbiol., 29: 370-374.