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Isolation of *C. Jejuni* and *Y. Enterocolitica* from Raw Chicken and Processed Chicken Products

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ABSTRACT

This study aimed to determine the role of chicken and chicken products in transmission of *Campylobacter jejuni* and *Yersinia enterocolitica;* major foodborne pathogens to consumers in Alexandria, Egypt. A total of 75 random samples of raw chicken and processed chicken products represented by chicken carcasses (thighs), chicken strips and chicken luncheon (25 of each) were collected from local markets in Alexandria Province for isolation and identification of *C. jejuni and Y. enterocolitica*. The results showed that the incidence of *C. jejuni* in the examined samples of chicken carcasses was 24 % while it could not be isolated from chicken strips and luncheon. In addition, it was recorded that the incidence of *Y. enterocolitica* in chicken carcasses was 32% while it could not be isolated from chicken strips and luncheon. Based on the obtained results of the current study, it was clear that raw chicken carcasses were contaminated with *C. jejuni and Y. enterocolitica* as compared to processed chicken products (chicken strips and luncheon) that may be traced back to cross contamination occurred during slaughtering, evisceration, transportation, and handling during retailing in the local markets with absence of awareness about different sources of contamination and measures of personal hygiene of sellers. Finally, the public health hazard of isolated bacteria, as well as the recommended measures to lower them in retailed raw chicken was discussed.

1. INTRODUCTION

Chicken and chicken products are very popular food in Egypt as well as throughout the world as they are delicious, nutritious and cheap source of animal protein. Historically, poultry meat products are developed to prolong the quality period of chicken meat for future use and to add varieties to consumers' diet. Poultry products are categorized as raw or processed products (Branscheid, 1993).

Chicken meat and products are contaminated from different sources starting from defeathering, evisceration and the subsequent during processing in plants (Levin et al., 2001). So, chicken meat was indicated as a potential source of the pathogenic bacteria including; *Campylobacter jejuni* and *Yersinia enterocolitica* that are among the principal causes of human gastroenteritis worldwide (EFSA, 2007).

Raw chicken is frequently considered to be an important source of *Campylobacter spp.* (Pearson et al., 2000), and specific campylobacteriosis outbreaks have been identified as being caused by chicken (Forbes, 2009). There are many reports describing Campylobacter contamination in retail poultry meats and/or products in the world including; Alter et al., (2004), Bohaychuk et al., (2006), Sallam, (2007), Madden et al., (2011) and Aliyu et al., (2012).

Also, chicken meat was a specific food category with respect to yersiniosis risk assessment. *Y. enterocolitica* is Gram-negative facultative anaerobic non-spore-forming straight rods or coccobacilli that belong to the family *Enterobacteriaceae* (Bottone et al., 2005). It was classified as bacterial food poisoning according to the duration of onset slow within 16-48 hours and it is the third most commonly reported zoonosis in Europe where 8,979 cases were reported in 2006 (Abu El-Naga et al. 2014). So, the objective of the present study was to determine the role of raw chicken and some processed chicken products in transmission of *C. jejuni* and *Y. enterocolitica* to consumers in Alexandria, Egypt.

2. MATERIALS AND METHODS:

2.1. Sampling:

A total of 75 random samples of raw chicken and processed chicken products represented by chicken carcasses, chicken strips and chicken

luncheon (25 of each) were collected from local markets in Alexandria Province for isolation and identification of *C. jejuni and Y. enterocolitica.* Each sample of chicken carcasses and chicken luncheon (250 g) was kept in separate sterile plastic bags while each sample of chicken strips was represented by one package. All samples were transferred directly with a minimum of delay to the laboratory of the Food Hygiene Department, Faculty of Veterinary Medicine, Alexandria University under possible aseptic conditions.

2.2. Isolation and identification of *C. jejuni* according to Corry et al., (2001):

About 25 g of each examined sample were transferred aseptically into a sterile homogenizer flask containing 225 ml Bolton broth, the samples were thoroughly blended for one minute at 14000 rpm, then the homogenates were incubated at 37 °C for 48 hours under microaerophilic conditions (10% O_2 ,5% CO_2 and 85% N_2). A loopful from homogenate tube was streaked onto Charcoal Cefoperzone Deoxycholate Agar (CCDA) and incubated under microaerophilic conditions (Gas pack jar) at 37 °C for 24 hours and for another 24 hours at 42 °C.

Typical colonies were smooth, convex and shiny grayish. The colonies were picked up and subjected to the following biochemical tests; catalase, oxidase, Indole production test, urease production, H_2S production and Hippurrate hydrolysis.

2.3. Isolation and identification of *Y. enterocolitica* according to Bercovier and Mollar, (1984):

Another 25 g of each sample were taken under aseptic conditions to sterile homogenizer flask containing 45 ml of sterile peptone water (0.1%). The contents were homogenized at 14000 rpm for 2.5 minutes. The mixture was allowed to stand for 10 minutes at room temperature then 1ml of supernatant was added to 5 ml of trypticase soya broth (TSB) (enrichment broth) and incubated at 25 °C for 24 hours. A loopful of enrichment was transferred to 0.1 ml of KOH (0.5%) in 0.5% saline for 2-3 seconds to suppress background flora after enrichment, then a loopful of enrichment was streaked to MacConkey's plates and incubated at 30°C for 1-2 days.

The lactose negatives colonies on MacConkey's agar plates were selected and streaked onto CIN (Cefsulodin, Irgasan, Novobiocin) Agar plates then were incubated at 30°C for 1-2 days. One to five susceptible colonies of typical "bull's eye" appearance (small and smooth, with a red center and translucent rim) on the CIN agar plates were individually isolated and subculture on nutrient agar for biochemical identification by catalase, oxidase, urease production, Simmon´s citrate, the behavior in TSI agar and sugar fermentation tests.

4. DISCUSSION

Campylobacter is found mostly in chicken meat with poultry and poultry farms playing a key role in the epidemiology of human infection. In the European Union, Campylobacter is still the most commonly reported cause of bacterial foodborne illness with a notification rate of 55.49 cases per 100,000 of population in 2012 (EFSA, 2012).

Poultry is a natural reservoir of Campylobacter species, constituting the most important source of human infection. The consumption of undercooked poultry meat or the mishandling of raw poultry products is considered to be the main risk factors associated with human campylobacteriosis (Kittl et al., 2013).

3. RESULTS AND DISCUSSION

Table (1): Incidence of *C. jejuni* and *Y. enterocolitica* in raw chicken and chicken products

Chicken samples / Enteric bacteria	Raw chicken (n=25)		Strips (n=25)		Luncheon (n=25)	
	No.	%	No.	%	No.	%
C. jejuni	6	24.0	0	0.0	0	0.0
Y. enterocolitica	8	32.0	0	0.0	0	0.0

It is evident from the results recorded in Table (1) that the incidence of *C. jejuni* in the examined samples of raw chicken was 24%. On contrary, it could not be isolated from the examined samples of processed meat products including chicken strips and luncheon.

This finding highlighted the absence of awareness about different sources of contamination of chicken carcasses during slaughtering, evisceration, washing, handling and retailing as compared to hygienic conditions during processing.

This result was lower than that reported by Sallam, (2007) who study the prevalence of *Campylobacter* in fresh chicken meat and found that 64.7% of the examined samples were contaminated with *Campylobacter* and 81.5% of isolates were identified as *C. jejuni* and Samaha et al., (2012) who detected *C. jejuni* in 76% of chicken meat and failed to isolate it from chicken nuggets and chicken luncheon.

Overall, raw poultry is recognized as a significant cause of human campylobacteriosis and Campylobacter is the most common cause of bacterial gastroenteritis. Also, the incidence of human campylobacteriosis is increasing worldwide (Sheppard, 2009). The incidence found in this study of raw chicken is cause for concern. There is a need for poultry producers supplying the retail market to introduce effective interventions to reduce the prevalence of this pathogen in final product.

As shown in Table (1), the incidence of *Y. enterocolitica* in the examined samples of raw chicken was 32%. On contrary, it could not be detected in chicken strips and luncheon. Isolation of *Y. enterocolitica* from examined raw chicken meat samples reflected contamination in working place, workers hands and cutting knife (Mahdavi et al., 2012).

This finding was agreed with Bonardi et al., (2010) who recorded an incidence of 32.5% while it was higher than that recorded by Momtaz et al., (2013) (18.33%) and Shabana et al., (2015) (17.5%). On contrary, it disagreed with Pavlovic et al., (2007); Mauro et al., (2008) and Anju et al., (2014) who could not isolate Y. enterocolitica in their examined fresh poultry meat samples. The contact with chicken faeces and lack of hygiene in chicken slaughterhouses are the two most frequent reasons in chicken meat contamination with *Y. enterocolitica,* which could easily spread and cause yersiniosis in humans (Momtaz et al., 2013).

The study concluded that high proportion of raw chicken meat marketed in Alexandria province are contaminated by *C. jejuni* and *Y. enterocolitica*, with a possible risk from such microorganism especially from consumption of undercooked or post-cooking contaminated chicken products. Raw retail meats are potential vehicles for transmitting food-borne diseases, and our findings stress the need for increased implementation of hazard analysis of critical control point (HACCP) and consumer food safety education efforts.

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