Isolation of Enterobacteriacaea from poultry products in El-Behera and Alexandria governorates

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A B S T R A C T

A grand total of 90 random samples of poultry meat products (chicken fillet, chicken thigh, chicken nuggets, chicken strips, chicken luncheon and chicken pane) 15 of each were collected from local slaughter poultry shops and different supermarkets in El-Behera and Alexandria governorates. All samples were examined bacteriologically for determination of aerobic plate count (APC), Enterobacteriaceae count, isolation and identification of Salmonella, *E. coli* and Shigella species. The results showed that the mean values of APC in the examined samples of chicken fillet, chicken thigh, chicken nuggets, chicken strips, chicken luncheon and chicken pane were $5.52\times 10^6 \pm 2.51 \times 10^6$, $1.87\times 10^6 \pm 0.53\times 10^6$, $5.10\times 10^4 \pm 1.70\times 10^4$, $9.50\times 10^4 \pm 5.20\times 10^4$, $2.15\times 10^5 \pm 6.53\times 10^4$ and $2.90\times 10^4 \pm 1.50\times 10^4$ cfu/g, respectively. While the mean values of Enterobacteriaceae count in the same examined samples were $1.19\times 10^5 \pm 0.50\times 10^5$, $7.18\times 10^4 \pm 4.34\times 10^4$, $5.80\times 10^2 \pm 3.50\times 10^2$, $7.50\times 10 \pm 4.70\times 10$, $3.80\times 10^3 \pm 2.50\times 10^3$ and $1.09\times 10^2 \pm 1\times 10^2$ cfu/g, respectively. On the other hand Salmonella organisms were isolated from chicken filet, chicken thigh and chicken nuggets with percentages of 13.33%, 33.33% and 6.67%, respectively. Moreover, the isolated Salmonellae could be serologically identified as *S. typhimurium, S. enteritidis, S. heidelberg, S. muenster, S. kentucky and S. anatum*. While, *E. coli* was isolated from the examined samples of chicken fillet and chicken thigh with percentages of 13.33% and 33.33%, respectively. Moreover, the isolated serotypes of *E. coli* were Enteropathogenic *E. coli* (O_{78}:k_{80}, and O_{55}:k_7), Enterotoxogenic E. coli (O_{125}:k_{21} and O_{127}:k_6), and Enterheamorrhagic *E. coli* (O_{26}:k_{11} and O_{111}:k_4). On the other hand, all the examined samples were free from *Shigella spp*.

Key words: Salmonella-Shigella –E.coli-poultry products -Enterobactereaceae

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1. INTRODUCTION

Poultry meat and its products are very popular food throughout the world and no wonder since They are delicious, nutritious and considered as a good and cheap source of protein characterized by good flavor and easily digested on the other hand, they rank first or second in foods associated with disease in most of the countries all over the world where USA ranked third of the reported food-borne disease outbreaks (Bean and Griffin, 1990).

Poultry products can be a route of introduction of pathogenic bacterium. Therefore, the microbial content of these products should be minimized for consumption (Carvalho et al., 2005). Processing of poultry products requires a severe microbiological quality control, considering they are one of the main sources of food borne infections.

Enterobacteriaceae family is a group of bacteria that is used to assess the general hygiene status of a food product (HPA, 2004). Where ever Salmonella was selected as the largest pathogenic microorganism because it is one of the most common causes of food poisoning, it

present at varying frequencies on all types of poultry products (Rose et al., 2002). Also, the presence of E. coli in food of animal origin is considered as indicator of faults during preparation, handling, storage or service (Tebbut, 1999). More ever Shigella infections remain a global public health concern, causing diarrhea in developing regions (Guerrant et al., 1990). Therefore, this study is designed to assess the contamination of some poultry products by Enterobacteriaceae.

2. MATERIAL AND METHODS

2.1. Collection of samples

A grand total of 90 random samples of poultry products classified into 30 samples of raw poultry products (chicken fillet and chicken thigh) (15 of each), 30 samples of half cooked poultry products (chicken nuggets and chicken strips) (15 of each) and 30 samples of cooked poultry products (chicken luncheon and chicken pane) (15 of each) were collected from local slaughter poultry shops and different supermarkets in El-Behera and Alexandria governorates and transferred as quickly as possible to the laboratory to be examined bacteriologically.

2.2.Preparation of samples for bacteriological examination (APHA, 1992)

Chicken fillet, chicken thigh and chicken luncheon samples were firstly cauterized by using hot spatula (surface sterilization) then the cauterized parts were removed by using sterilized scalpel and forceps, while the chicken nuggets, chicken strips and chicken pane samples were firstly thawed by holding in refrigerator at 3-4^{0}C for 1 hour. Then under complete aseptic conditions 25 grams of each sample were weighted and transferred into a sterile homogenizer flask containing 225 ml of sterile peptone water 0.1%. and homogenized at 2000 r.p.m for 1-2 minutes then tenth - fold serial dilutions were prepared.

2.3. Determination of APC (APHA, 1992)

by using standard plate count agar media.

2.4.Determination of Enterobacteriaceae count (ISO, 2004)

by using violet red bile glucose agar media (VRBG).

2.5.Isolation and Identification of Salmonella (ISO, 2002)

by using Rappaport Vassilidis broth and Xylose Lysine Desoxycholate (XLD) agar.

2.6. Isolation and Identification of E. coli (APHA, 1992)

by using MacConkey broth and Eosin Methylene Blue (EMB) agar.

2.7. Isolation and Identification of Shigella (Feng et al., 2007)

by using Rappaport vassilidis broth and Salmonella Shigella (SS) agar.

3. RESULTS

Table (1) reported that the APC (cfu/g) in the examined samples varied from 1.20×10^5 to 2.96×10^7 with an average value of $5.52\times 10^6 \pm 2.51 \times 10^6$ for chicken fillet, from 2.80×10^5 to 7.10×10^6 with an average value of $1.87\times 10^6 \pm 0.53\times 10^6$ for chicken thigh, from $3.2.\times 10^3$ to 2.32×10^5 with an average value of $5.10\times 10^4 \pm 1.70\times 10^4$ for chicken nuggets, from 3×10^3 to7.8×10^5 with an average value of $9.50\times 10^4 \pm 5.20\times 10^4$ for chicken strips, from 3.60×10^3 to 8.80×10^5 with an average value of $2.15\times 10^5 \pm 6.53\times 10^4$ for chicken luncheon and from 1.50×10^3 to 2.20×10^5 with an average value of $2.90 \times 10^4 \pm 1.50 \times 10^4$ for chicken pane respectively. The differences between the examined samples of poultry products were significant ($P \leq 0.01$). Results given in table (2) showed that the total Enterobacteriacae count (cfu/g) in the examined samples ranged from 1.90×10^3 to 7.20×10^5 with an average value of

$1.19\times 10^5 \pm 0.50\times 10^4$ for chicken fillet, 1.00×10^2 to 6.20×10^5 with an average value of $7.18\times 10^5 \pm 4.34\times 10^4$ for chicken thigh, 5×10 to 4.50×10^3 with an average value of $5.80\times 10^2 \pm 3.50\times 10^2$ for chicken nuggets, 1×10^2 to 6×10^2 with an average value of $7.50\times 10 \pm 4.70\times 10$ for chicken strips, 5×10 to 2.89×10^4 with an average value of $3.80\times 10^3 \pm 2.50\times 10^3$ for chicken luncheon and 1×10 to 1.52×10^3 with an average value of $1.09\times 10^2 \pm 1\times 10^2$ for chicken pane, respectively.The differences between the examined samples of poultry products were significant ($P \leq 0.05$). Regarding the results in table (3), the incidences of isolated *Salmonellae* were 13.33% and 33.33% of the examined chicken fillet and chicken thigh samples, respectively, while *Salmonellae* could not detected in all heat treated chicken meat products except in chicken nuggets in a rate of 6.67%. Table (4) reported that Salmonellae could be identified serologically as *S. typhimurium* (13.33%), *S. enteritidis* (13.33%), *S. heidelberg* (6.67%), *S. muenster* (6.67%), *S. kentucky* (6.67%) and *S. anatum* (6.67%). Results achieved in Table (5) indicated that *E.coli* was isolated with incidences of 13.33% and 33.33% in chicken fillet and chicken thigh samples respectively, but could not be Isolated from heat treated chicken meat products. Regarding the results in table (6), the incidence of serologically identified *E. coli* as Enteropathogenic *E. coli* (O_{78}:k_{80} and O_{55}:k_7) was 13.33%, Enterotoxogenic *E. coli* (O_{125}:k_{21} and O_{127}:k_6) was 13.33%, and Enterheamorrhagic *E. coli* (O_{26}:k_{11} and O_{111}:k_4) was 13.33%. Results achieved indicated that Shigella spp. Failed to be detected in all the examined raw, half cooked and full cooked chicken products.

Table (1): Statistical analytical results of Aerobic Plate Count (APC) (cfu/g) in the examined chicken meat samples (n=15).

Samples	Min.	Max.	Mean ± S.E*
A-Row products			
Fillet	1.2×10^5	3.0×10^7	$5.5\times 10^6 \pm 2.5\times 10^{6+}$
Thigh	2.8×10^5	7.1×10^6	$1.9\times 10^6 \pm 5.3\times 10^5$
B- Half cooked			
Nuggets	3.2×10^3	2.3×10^5	$5.1\times 10^4 \pm 1.7\times 10^4$
Chicken strips	3.0×10^3	7.8×10^5	$9.5\times 10^4 \pm 5.2\times 10^4$
C-Cooked			
Luncheon	3.6×10^3	8.8×10^5	$2.2\times 10^4 \pm 6.5\times 10^4$
Pane	1.5×10^3	2.2×10^5	$2.9\times 10^4 \pm 1.5\times 10^4$

S.E* = standard error of mean + = Significant differences between products ($P<0.01$)

Table (2): Statistical analytical results of total Enterobacteriaceae counts/gm in the examined chicken meat samples (n=15).

Samples	Min.	Max.	Mean ± S.E*
A-Row products			
Fillet	1.90×10^3	7.20×10^5	$1.2\times 10^5 \pm 4.97\times 10^4$
Thigh	1×10^2	6.20×10^5	$7.18\times 10^4 \pm 4.34\times 10^4$
B- Half cooked			
Nuggets	5×10	4.50×10^3	$5.81 \times 10^2 \pm 3.55\times 10^2$
Chicken strips	1×10^2	6×10^2	$7.5\times 10^1 \pm 4.7\times 10^1$
C-Cooked			
Luncheon	5×10	2.89×10^4	$3.80\times 10^3 \pm 2.46\times 10^3$
Pane	1×10	1.52×10^3	$1.09\times 10^2 \pm 1.01\times 10^2$

S.E* = standard error of mean + = Significant differences between products (P<0.05)

Table (3): Incidence of Salmonella in the examined chicken meat samples (n=15).

Samples	Positive Samples	
	No	%
A-Row products		
Fillet	2	13
Thigh	5	33
B- Half cooked		
Nuggets	1	7
Total	8	53

Table (4): Serotyping of Salmonella isolated from the examined chicken meat samples (n=15).

Identified Strains	Fillet		Thigh		Nuggets		Total	
	No.	%	No.	%	No.	%	No.	%
Salmonella Typhimurium	1	7	1	7	--	--	2	13
Salmonella Enteritidis	1	7	1	7	--	--	2	13
Salmonella Heidelberg	--	--	1	7	--	--	1	7
Salmonella Kentucky	--	--	1	7	--	--	1	7
Salmonella Muenster	--	--	1	7	--	--	1	7
Salmonella Anatum	--	--	--	--	1	7	1	7
Total		13	5	33	1	7	8	53

Table (5): Incidence of E. coli in the examined chicken meat samples (n=15)

Samples	Positive Samples	
	No	%
A-Row products		
Fillet	2	13
Thigh	5	33
Total	7	46

Table (6): Serotyping of E. coli isolated from the examined chicken meat Sample (n=15).

Identified Strains	Fillet		Thigh		Types	Total	
E coli O_{78}:k_{80}	--	--	1	7			
E coli O_{55}:k_7	1	7	--	--	EPEC	2	13
E coli O_{125}:k_{21}	1	7	--	--			
E coli O_{127}:k_6	--	--	1	7			
E coli O_{26}:k_{11}	--	--	1	7	ETEC	2	13
E coli O_{111}:k_4	--	--	2	13			
Total	2	13	5	33	EHEC	3	20

EPEC: Enteropathogenic *E. coli* ETEC: Enterotoxigenic *E. coli* EHEC: Enterohaemorrhagic *E.*

4. DISCUSSION

Poultry meat products are subjected to the risk of contamination with various pathogens from different sources, primary during pre-processing and processing steps and secondary after processing through packaging, marketing and storage. Such contamination may render these food articles unfit for human consumption or even harmful to consumers.

The level of APC and Enterobacteriaceae count in poultry products can be routinely used as indicators of improper hygiene during processing and incorrect storage conditions, which can lead to proliferation of pathogens and toxin production (Zweifel *et al*., 2005).

Higher APC in chicken meat cuts were obtained by Huong *et al*. (2009) ($47.8\times 10^6 \pm 0.18\times 10^6$). While, nearly similar results for chicken cuts were obtained by AL-Dughaym and Altabari (2010) (6.2×10^6 for fillet and 5.1×10^6 for thigh), Saikia and Joshi (2010) (1.07×10^6 for thigh). As well as, lower APC in chicken meat were obtained by Ruban and Fairoze (2011) (2.18×10^5 for thigh and 1.78×10^5 for brest).

Higher results for heat treated chicken meat products were obtained by El-Deeb *et al*. (2011) ($7.5\times 10^5 \pm 2.6\times 10^4$ for luncheon and $7.1\times 10^5 \pm 1.6\times 10^4$ for nuggets). As well as, nearly similar results were obtained by Noha and

Gehad (2005) ($7.4\times 10^4 \pm 1.8\times 10^4$ for strips).

Higher APC in chicken filet than in chicken thigh was due to processing of breast into parts with removal of the skin, soaking chicken fillet in unclean water to increase their weight, using unclean knives and chopping tables which manufactured from wood. All these factors lead to further spread of contamination to the fleshy parts.

Nearly similar Enterobactereacaea count in chicken meat cuts were obtained by Saikia and Joshi (2010) (2×10^4 for thigh) and El-Deeb *et al*. (2011) ($2.5\times 10^5 \pm 0.6 \times 10^4$ for fillet), lower Enterobactereacaea in chicken meat were obtained by Nawar (2007) (7.12×10^3 for thigh).

Salmonella organisms were previously isolated by Nawar (2007) (11.11 for thigh), Ruban and Fairoze (2011) (71.43 for thigh), Samaha et al. (2012) (8% for nuggets) and Ragy et al. (2011) (16% for fillet). In contrary to our results the isolation of Salmonella from pane and luncheon was reported by Samaha et al. (2012) (12% and 8%) respectively.

The presence of *Salmonella* in chicken meat may be attributed to contamination during slaughtering and/or processing from workers' hands (Carraminana *et al*., 1997).

In this study, *E.coli* could not be found in chicken meat products due to heat treatment or/and freezing. (Abd El - Haffeiz 1999). Nearly similer results were obtained by Ouf-Jehan (2001). On the other hand, El-Tahan *et al*. (2006) isolated *E.coli* only from both nuggets and luncheon samples collected from Down Town retail markets but samples from Shubra and Nasr city were free.

The presence of *E. coli* in chicken fillet and thigh may be attributed to contamination during handling and processing and because such samples are raw not frozen or heat treated.

The presence of *E.coli* in the examined chicken products considered as indicator for improper handling or unhygienic conditions (Hashim, 2003).

Shigella was transmitted through the fecal-oral route, with the majority of illnesses arising through the consumption of fecally contaminated food and water. Poor personal hygiene and sanitation are the common sources of such food contaminations (Sapsford *et al*., 2004).

The obtained results concluded that the chicken fillet were more contaminated than chicken thigh, while the incidence of Salmonella spp. and *E. coli* in chicken thigh was higher than in chicken fillet.

Escherichia coli failed to be detected in all the examined heat treated chicken samples while Salmonella was detection in only one sample. So, it is uncertain whether inadequate cooking (microwave oven) or the presence of pathogenic bacteria with elevated thermal resistance is the more likely cause of human illness associated with these products. Moreover, food born infection due to members of Shigella spp. may not be as frequent as those caused by other food borne pathogens. Results achieved indicated that *Shigella spp.* failed to be detected in all the examined raw, half and cooked chicken products. Cardoso *et al.* (2006) isolated *Shigella* from fresh and refrigerated poultry products, but failed to detect *Shigella* in frozen samples. *Shigella* species are highly sensitive and die rapidly in unfavorable environments including the unavoidable temperature fluctuations encountered during transport. A significant problem in elucidating the potential hazard of non-culturable pathogenic bacteria is the inability to detect such cells in the natural

environment by routine bacteriological culture methods. *Shigella* species can exist in the viable but non-culturable (VBNC) state. Polymerase chain reaction (PCR), a highly-selective and sensitive method, can detect VBNC *Shigella* DNA in laboratory microcosms (Von Seidlein *et al.* 2006).

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عزل الميكروبات المعوية في منتجات الدواجن في محافظة البحيرة والاسكندرية

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الملخص العربي

للوقوف على مدي سلامة منتجات لحوم الدجاج. تم جمع (90) عينة عشوائية من ستة انواع مختلفة من منتجات لحوم الدواجن والتي تراوحت من مجتزئات (فيليه الصدور والأوراك) الي المصنعات (لنشون، بانيه، ناجتس، ستربس) من اسواق مختلفة بمحافظة البحيره والاسكندرية (بمعدل 15 لكل نوع) حيث أجريت الفحوص البكتريولوجية عليها لتحديد العد الكلي البكتيري، الميكروبات المعوية وكذلك عزل ألأيشريشيا كولاي والسالمونيلا والشيجيلا بالطرق التقليدية وقد أظهرت النتائج أن متوسط العد الكلى للميكروبات الهوائية لعينات فيليه، اوراك، ناجتس، ستربس، لنشون وبانيه الدجاج علي التوالى 5.52× 10^{6} ± 2.51 × 10^{6}، 1.87× 10^{6} ±5.27× 10^{5}، ±10^{4}5.11×10^{4} 1.74× 10^{4}، 9.51× 10^{4}±5.16× 10^{4} ، 2.15× 10^{5}±6.53 × 10^{4} و ±10^{4}2.85×10^{4} 1.49×10^{4}/ جم.

بينما كان متوسط العد الكلى للميكروبات المعوية لعينات فيليه، اوراك، ناجتس، ستربس، لنشون ا وبانيه الدجاج علي التوالى 4.97 ×10^{4}± 1.19 ×10^{5}، ±10^{4}7.18×10^{4} 4.34×10^{4}، ±10^{2}5.81×10^{2} 3.55× 10^{2}، 10^{1}±4.49×10^{1}7.47×، 10^{3}±3.80×10^{3}2.46×10^{3}و 10^{3}±1.01×10^{2}1.09×/ جم.

وقد تم عزل ميكروبات السالمونيلا من عينات فيليه، اوراك وناجتس الدجاج بنسب 13%، 33%و 7% على التوالى.وبالفحص السيرولوجى تبين أن العترات المعزولة هى: *S. Typhimurium* ,

S Enteritidis.,S. Heidelberg, S. Muenster, S. Kentucky and S. Anatum

كما تم عزل ميكروب الأيشريشيا كولاي من فيليه واوراك الدجاج بنسبة 13% و33% على التوالى وكانت العترات المعزولة هي.*O26:k11, O78:k80 , O55:k7, O111:k4 ,O125:k21 ,O127:k6*

وقد تم دراسة ومناقشة الأهمية الصحية للميكروبات المعزولة ومصادر تلوث الدواجن ومنتجاتها التي تم فحصها بالإضافة إلي اقتراح التوصيات اللازمة لسلامه هذه المنتجات.

(مجلة بنها للعلوم الطبية البيطرية: عدد 27(1):109- 117, سبتمبر 2014)