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Incidence of Enteric Pathogens in Cooked Poultry Products in Relation to Public Health in Alexandria Province

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ABSTRACT

A total of 200 random samples of processed chicken meat products including frankfurter, luncheon, strips, and nuggets (50 of each) were collected from different supermarkets at Alexandria province. The samples were examined bacteriologically immediately after arrival to the laboratory for isolation and identification of some pathogenic bacteria that may contaminate these products as *E. coli*, *Salmonellae*, *Campylobacter jejuni*, and *Staphylococcus aureus*. The obtained results revealed that the incidence of *E. coli* in the examined samples of frankfurter, luncheon, strips, and nuggets was 10, 14, 6 and 0%, respectively. It was observed that the highest incidence was recorded in luncheon followed by frankfurter and lastly nuggets, the incidence of *Salmonellae* in the examined samples was 4, 6, 0 and 0%, respectively. The incidence of *Y. enterocolitica* in the examined samples of frankfurter, luncheon, strips, and nuggets was 4, 8, 2, and 2%, respectively. The incidence of *C. jejuni* in the examined samples of frankfurter, luncheon, strips, and nuggets was 6, 8, 0, and 2%, respectively. The incidence of *S. aureus* in the examined samples of frankfurter, luncheon, strips, and nuggets was 10, 8, 6, and 6%, respectively. Based on the recorded results in the current study, it was clear that the rates of isolation of the investigated enteric bacteria were higher in chicken frankfurter and luncheon as compared to strips and nuggets that may be attributed to the hygienic conditions of the working places and the awareness of the workers.

1. INTRODUCTION:

Chicken products contain an abundance of all nutrients required for the growth and multiplication of most microorganisms. Adequate quantities of these constituents exist in Chicken products in available form, so good manufacturing practices and the hygienic conditions of these products are very important during the steps of preparation, handling, and storage, as they are contaminated from different sources. These may lead to spoilage of these products and/or act as public health hazard to consumer.

Chicken meat products are very popular food in Egypt as well as throughout the world. No wonder since it's delicious as considered as a good and cheap source of protein characterizes by good flavor and easily digested. The increase of human population and the great progress of various aspects of life make the consumer to use meat products in different forms for its ease in preparation as luncheon, frankfurter, raw sausage and pasterma.

Many human foodborne illnesses can be caused by consumption of foodstuffs (including chicken meat products) contaminated with pathogenic bacteria from animal intestinal contents or hides. Steps that have been taken in the slaughter plant to decrease the spread of foodborne pathogenic bacteria (e.g., hazard analysis and critical control point methods) have been very effective; however, chicken meat products are still the source of foodborne bacterial human illnesses (Callaway et al., 2004).

Bacterial enteric pathogens were estimated to cause about 5 million illnesses, 46,000 hospitalizations, and 1458 deaths in the United

States each year. Food-producing animals (e.g., cattle, chickens, pigs, and turkeys) were the major reservoirs for many of these organisms, which include *Campylobacter* species and non-Typhi serotypes of *Salmonella enterica*, Shiga toxin–producing strains of *Escherichia coli* and *Yersinia enterocolitica* (Mead et al., 1999).

Numerically, the most important agents are *Salmonellae* and *Campylobacter* spp. Data for the European Union (EU) show that in 2001, there were 157822 reported cases of human salmonellosis and 156232 cases of *Campylobacter* enteritis (Cavitte, 2003), although both diseases are known to be under reported, the true figures are likely to be considerably higher and poultry by no means the most common source of these organisms.

E. coli is a normal inhabitant of the intestinal tract of humans and worm-blooded animals, and meat is a common source of *E. coli* contamination, which may be acquired during slaughter through fecal contact besides some pathogenic strains are responsible for enteric and diarrheal diseases, and they have been increasingly recognized as the most important causes of food borne diseases and outbreak all over the world (Bettelheim and Goldwater, 2014).

Salmonella is a member of the *Enterobacteriaceae,* Gram negative, motile, with peritrichous flagella and non-spore forming rods. Also, *Salmonella* is a facultative anaerobic (can grow with or without oxygen) catalase positive and oxidase negative bacteria. However, *Salmonella* is not included in the group of organisms referred to as coliforms (Lawley et al., 2008). More than 2,500 different types of *Salmonella* exist, some of which cause illness in both animals and people. Some types may cause illness in animals but not in people. Some serotypes are only present in certain parts of the world (Brands, 2006).

Campylobacter species are common bacterial pathogens associated with human gastroenteritis worldwide. In North America, Europe, and Japan, campylobacteriosis is one of the leading food-borne bacterial illnesses and the consumption of poultry meats and/or by-products is suspected a major cause of the illness. Most retail poultry meats and by-products in most of the countries were contaminated with *Campylobacter spp.* where *C. jejuni* was usually the dominant *Campylobacter* species isolated from retail poultry and *C. coli* was less frequently isolated (Suzuki and Yamamoto, 2009). The consumption of undercooked poultry meat or the mishandling of raw poultry products is the main risk factors associated with human campylobacteriosis (Kittl et al., 2013).

Staphylococcus aureus usually acts as a commensal bacterium, asymptomatically colonizing about 30% of the human population; it can sometimes cause disease and it has been implicated in cases of severe diarrhea as well as it may be one of the main causes of food poisoning gastroenteritis among consumers (Tong et al., 2015).

Therefore, great emphasis is being placed on the microbiological aspects of poultry carcasses and on searching for alternative mechanisms to reduce both natural and cross contamination, thus avoiding major public health problems so it is important to adopt hazard analysis and critical control point principles in production, processing, and handling of poultry carcasses to achieve pathogen free products.

So, the present work was carried out to determine the incidence of enteric pathogens in cooked poultry products in relation to public health in Alexandria Province.

2. MATERIALS AND METHODS:

2.1. Samples:

A total of 200 random samples of processed chicken meat products including frankfurter, luncheon, strips, and nuggets (50 of each) were collected from different supermarkets at Alexandria province. Each sample was kept in a separate plastic bag and transferred directly with a minimum of delay to the laboratory of Food Hygiene Department, Faculty of Veterinary Medicine, Alexandria University in an insulating refrigerated container under complete aseptic condition to avoid any changes in the quality of the sample.

2.2. Preparation of samples for bacterial isolation:

It was performed according to the procedures describe by APHA, (2001). 25 g of each sample were aseptically transferred into sterile blender flask containing 225 ml of sterile peptone water 1% and homogenized at 14000 rpm for 2.5 minutes.

2.3. Bacterial detection:

2.3.1. Isolation, identification and serotyping of *E. coli*:

It was carried on specific media then the isolates were confirmed to be *E. coli* by various biochemical assays, as per Bergey's manual of determinative bacteriology (Holt et al., 1994). The

serological identification of isolates was carried out according to Varnam and Evans, (1991). Isolated strains of *E. coli* were identified serologically using diagnostic Sera (Denka Seiken Co., LTD, Tokyo, Japan).

2.3.2. Isolation, identification and serotyping of *Salmonellae*:

Salmonella cultures from all samples were performed according to ISO, (2002). 0.1 ml from each BPW tube (after incubation) was transferred into a 10 ml Rappaport-Vasilliadis broth (RV broth, Difco, USA) and incubated at 42 °C for 24-48 hours. The RV broth samples were streaked onto Xylose- Lysine-Desoxycolate agar (XLD, oxoid) plates and incubated overnight at 37 °C. Typical colonies were picked and further tested by standard biochemical methods. Full identification of the *Salmonella* suspect isolates were done after matching the achieved morphological, biochemical, and serological results against standard methods reported by Garrity (2001). The serological identification of the strains was carried out with *Salmonella* polyvalent O and H antisera in the Clinical Microbiology Department, Central Health Laboratories of Ministry of Health on Egypt.

2.3.3. 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.

2.3.4. Isolation and identification of *S. aureus*:

It was carried out according to per Bergey's manual of determinative bacteriology (Holt et al., 1994). Screening for pathogenic *S. aureus* was done by performing various biochemical assays, including Coagulase test, DNase test (Baird, 1996) and Thermostable nuclease test (TNase) (Lachica et al., 1971).

3. RESULTS AND DISCUSSION

Chicken and chicken meat 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 its products are contaminated from different sources starting from defeathering, evisceration and the subsequent during processing in plants (Levin et al., 2001).

Poultry are the most common food vehicle of human infection with bacterial pathogens throughout the world. So, chicken meat was indicated as a potential source of the pathogenic bacteria including *E. coli, Salmonellae, Campylobacter jejuni* and *Yersinia enterocolitica* that are among the principal causes of human gastroenteritis worldwide (EFSA, 2007).

The isolation of pathogenic *E. coli* from food indicated contamination with fecal matter of animal and human origin (Manna et al., 2008). Food products that show evidence of fecal contamination were generally regarded as a greater risk to human health, as they were more likely to contain human-specific enteric pathogen. Some strains of *E. coli* could cause food borne disease, ranging from mild enteritis to serious illness and death (WHO, 1997).

It was evident from Table (1) that the incidence of *E. coli* in the examined samples of frankfurter, luncheon, strips, and nuggets was 10, 14, 6 and 0%, respectively. It was observed that the highest incidence was recorded in luncheon followed by frankfurter and lastly nuggets.

Table (1): Occurrence of *E. coli* in cooked chicken products

Cooked chicken products (n= 50 of each)	*E. coli*	
	Positive	%
Frankfurter	5	10.0
Luncheon	7	14.0
Strips	3	6.00
Nuggets	0	0.00

Table (2): Serotyping of Enteropathogenic *E. coli* isolated from cooked chicken products

Cooked chicken products / *E. coli* Serotypes	Frankfurter (n= 50)		Luncheon (n= 50)		Strips (n= 50)	
	No.	%	No.	%	No.	%
O_{78}	1	2.0	2	2.0	0	0.0
$O_{111}:H_4$	1	2.0	1	2.0	1	2.0
O_{124}	1	2.0	2	4.0	0	0.0
$O_{55}:H_7$	2	4.0	0	0.0	1	2.0
O_{142}	0	0.0	1	2.0	0	0.0
$O_2:H_6$	0	0.0	1	2.0	1	2.0
Total	**5**	**10.0**	**7**	**14.0**	**3**	**6.0**

Table (3): Occurrence of *Salmonellae* in cooked chicken products

Cooked chicken products (n= 50 of each)	*Salmonellae*	
	Positive	%
Frankfurter	2	4.0
Luncheon	3	6.0
Strips	0	0.0
Nuggets	0	0.0

Table (4): Serotyping of *Salmonellae* isolated from cooked chicken products.

Cooked chicken products / *Salmonellae* Serotypes	Frankfurter (n= 50)		Luncheon (n= 50)	
	No.	%	No.	%
S. Enteritidis	1	2.0	1	2.0
S. Typhimurium	1	2.0	1	2.0
S. Kentucky	0	0.0	1	2.0
Total	2	4.0	3	6.0

Table (5): Occurrence of *Campylobacter jejuni* in cooked chicken products

Cooked chicken products (n= 50 of each)	*Campylobacter jejuni*	
	Positive	%
Frankfurter	3	6.0
Luncheon	4	8.0
Strips	0	0.0
Nuggets	1	2.0

Table (6): Occurrence of *Staphylococcus aureus* in cooked chicken products

Cooked chicken products (n= 50 of each)	*Staphylococcus aureus*	
	Positive	%
Frankfurter	5	10.0
Luncheon	4	8.0
Strips	3	6.0
Nuggets	3	6.0

Nearly similar results were obtained by Hemeda, (2017) who recorded that the incidence of *E. coli* in the examined samples of luncheon was 16% and El Ramy, (2017) who found that the incidence of *E. coli* in strips and luncheon (processed chicken products) was 12 and 16%, respectively. On contrary, it was lower than that recorded by Rady et al. (2011) who recorded that the incidence of *E. coli* in chicken luncheon was 24 % and Sharaf and Sabra (2012) who recorded that the incidence of *E. coli* in chicken luncheon was 25%. On the other hand, these results were higher than that recorded by Samaha et al. (2012) who could isolate *E. coli* with an incidence of 8 % in chicken luncheon.

There are two types of Enteropathogenic *E. coli* (EPEC): typical, which possess the EPEC adherence factor (EAF) plasmid and Atypical, which do not possess the EAF plasmid. Currently, the EPEC isolated in industrialized countries are atypical while those from developing countries are typical (Cheasty, 2008). Generally, the presence of *E. coli* in examined chicken products considered as an indicator for improper handling or unhygienic conditions which agreed with Hashim, (2003).

Serotyping of the obtained isolates of Enteropathogenic *E. coli* was tabulated in Table (2). It revealed the detection of O_{78} serotype (EHEC) in the examined samples of frankfurter and luncheon, with an incidence of 2 % for each, O_{111}:H_4 serotype (EIEC) in the examined samples of luncheon, frankfurter, and strips with an incidence of 2 % for each, O_{124} serotype (EPEC) in the samples of frankfurter and luncheon with an incidence of 2 and 4%, respectively, O_{55}:H_7 serotype (ETEC) in the samples of frankfurter and strips with an incidence of 4 and 2 %, respectively, O_{142} serotype (EPEC) in the samples of luncheon with an incidence of 2 % and O_2:H_6 serotype (ETEC) in the samples of luncheon and strips with an incidence of 2 % for each.

The obtained results were in harmony with those of Ibrahim et al., (2014) who identified 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) and Hemeda, (20117) who could isolate O_{111}:k_{58}, O_{124}:K_{72}, O_{26}: K_{60}, O_{128}:K_{67} and O_{86}: K_{61} strains at different rates from the examined samples of chicken luncheon. Also, they agreed with Osaili et al., (2014) who could not isolate *E. coli* O_{157}:H_7.

The presence of *E. coli* in the examined chicken products may be attributed to the food stands are simple structures where running water, toilets and washing facilities are seldom available. The washing of hands, utensils and dishes are often done in bowls or pots of water. Also, the disinfection is seldom carried out and pests may be attracted to vending sites if there is inadequate sewage disposal. Furthermore, foods prepared at these sites pose health risks as they are ineffectively refrigerated, and hygiene principles are not applied properly. Moreover, it may be due to handlers contaminate food via manual contact or via the respiratory tract by coughing and sneezing and contamination occurs also after heat treatment of the food (EFSA, 2014).

Salmonellae were one of the most frequent causes of food borne illness worldwide and transmission involves foods of animal origin (Khaitsa et al., 2007). The presence of *Salmonellae* in cooked foods is often attributed to inadequate sanitation, poor personal hygiene during food handling, processing and storage, presence of waste close to food preparation and food premises, and inadequate refrigeration. Proliferation of this organism in foods may, result from handling cooked foods by workers who are carriers of *Salmonellae* (Abdel Fattah, 2014).

Incidence of *Salmonellae* was tabulated in Table (3) and it revealed that the incidence of *Salmonellae* in the examined samples of frankfurter, luncheon, strips, and nuggets was 4, 6, 0 and 0%, respectively. These results agreed with Rady et al., (2011), Samaha et al., (2012) and Hemeda, (2017) who could isolate *Salmonellae* from chicken luncheon with an incidence of 20, 8 and 4%, respectively while they disagreed with Hamad, (2017) and El Ramy, (2017) who could not isolate *Salmonellae* from the examined samples of processed chicken products.

Serological identification of the obtained isolates of *Salmonellae* was presented in Table (4). It was noticed that *S. Enteritidis* in the examined samples of frankfurter and luncheon with an incidence of 2 % for each, *S. Typhimurium* in the examined samples of frankfurter and luncheon with an incidence of 2 % for each and *S. Kentucky* the examined samples of luncheon with an incidence of 2 %.

These results were in agreement with that of Antunes et al., (2003) who found that *S. Enteritidis* and *S. Hadar* were the most prevalent serotypes contaminating poultry products, Ibrahim et al., (2014) who found that the isolated *Salmonellae* were serologically identified as S. *Typhimurium, S. Enteritidis, S. Heidelberg, S. Muenster, S. Kentucky* and *S. Anatum,* Eskander, (2015) who observed that the most prevalent serotypes were *S. Enteritidis* (41.8%), *S. Typhimurium* (48.85%), *S. Virchow* (4.65%) and *S. Kentucky* (4.65%) and Hemeda, (2017) who identified *S. Enteritidis, S. Typhi* and *S. Paratyphi* from the examined samples of chicken fillet, luncheon and frankfurters. The isolation of *Salmonellae* from chicken meat may be attributed to contamination during slaughtering and / or processing from workers' hands. No detection of *Salmonella* in ready-to-eat food was the only satisfactory result (EFSA, 2014).

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).

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 reported cause of bacterial foodborne illness with a notification rate of 55.49 cases per 100,000 of population in 2012 (EFSA, 2012).

It is evident from the results recorded in Table (5) that the incidence of *C. jejuni* in the examined samples in the examined samples of frankfurter, luncheon, strips, and nuggets was 6, 8, 0, and 2%, respectively. These results disagreed

with Samaha et al., (2012) who failed to isolate it from chicken nuggets and chicken luncheon and El Ramy, (2017) who could not isolate *C. jejuni* from the examined samples of processed chicken products including strips and 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).

Staphylococcus aureus is a facultative anaerobic, gram-positive coccal non-motile and does not form spores. S. aureus appears as staphylococci (grape-like clusters) when viewed through a microscope, and has large, round, golden-yellow colonies, often with hemolysis, when grown on blood agar plate. It usually acts as a commensal bacterium, asymptomatically colonizing about 30% of the human population; it can sometimes cause disease (Tong et al., 2015).

S. aureus could be carried on human hands, nasal passage, or throats. Most food borne illness outbreaks were resulted of contamination from food handlers and production of heat stable toxins in the food. Sanitary food handling, proper cooking and refrigerating should prevent *S. aureus* food borne illness (FSIS, 2003).

As shown in Table (6), it was observed that the incidence of *S. aureus* in the examined samples in the examined samples of frankfurter, luncheon, strips, and nuggets was 10, 8, 6, and 6%, respectively. This result agreed with Mousa et al., (2014) who found that 80% of the examined luncheon samples were contaminated with *S. aureus*.

The detection of *S. aureus* in food products was a matter of concern as it was a pathogenic strain which could cause food poisoning due to the heat stable *Staphylococcus* enterotoxin which is resistant to gastrointestinal enzymes. Also, it was one of the most common causes of boils, impetigo, and folliculitis and in some cases, bacteremia and infections of the bones and wounds (Herman et al., 2011).

5. CONCLUSION:

According to the recorded results in the present work, it was clear that the rates of isolation of the investigated enteropathogenic bacteria were significant that may be attributed to the hygienic conditions of the working places and the awareness of the workers in addition the role of insufficient heat treatment of the processed products could not be neglected.

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