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Cooked poultry meat and products as a potential source of some food poisoning bacteria

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***Abstract**: This study was conducted in Assiut, Egypt, to investigate the prevalence rate of Listeria* spp., *Staph. aureus, Salmonella spp. and Campylobacter spp. in ready to eat (RTE) chicken meat and products. A total of 135 RTE chicken samples comprised of 50 chicken frankfurters, 35 chicken shawerma, 25 chicken breast and 25 chicken thigh which were collected randomly from different restaurants. The achieved results declared that Staph. aureus was the most predominant one; isolated from 22 (16.3%) from examined samples followed by L. monocytogenes was detected in 9 (6.7%) in cooked chicken samples. Salmonella was isolated from 6 (4.4%). The most prominent Salmonella serovars were S. Enteritides while, Campylobacter spp. was isolated from 3 (2.2%) of the samples. C. jejuni was the only strain isolated. Conclusion: RTE cooked chicken meat and products can be contaminated with a wide variety of pathogenic food poisoning microorganisms as Staph. aureus, L. monocytogenes, S. Enteritidis, S.Typhimurium and C. jejuni during processing, so it could be considered as an important public health risk. These results signify the importance of sustained surveillance of foodborne pathogens in cooked chicken meat to minimize the risk of contamination and protecting consumers against outbreaks of food poisoning.*

***Keywords**: RTE cooked chicken meat, L. monocytogenes, Staph. aureus, Salmonella spp., Campylobacter spp.*

I. Introduction

Ready-to-Eat (RTE) poultry meat and products are growing in popularity, with consumers opting for greater convenience. Alongside this trend, demands for its easy digestibility, a nutritious, safe and healthy food, which is low in fat and cholesterol compared to other meats but an excellent source of protein. Unfortunately, it could be contaminated with a variety of potentially pathogenic food borne pathogens that may cause human illness such as *Salmonella*, *Campylobacter*, *Staph. aureus* and *L. monocytogenes* (Daçi *et al*, 2016)**.** Chicken frankfurters are emulsion type cooked sausages which are very popular and highly consumed meat product in many countries (Özvural and Vural, 2008). Frankfurters are economical because of the use of meat by-products like skin and mechanically deboned poultry meat (MDPM) for the preparation because of the low cost of this ingredient (Feiner, 2006). Moreover, chicken frankfurters are flavored with spices and smoke application (Gonzalez-Vinas *et al*., 2004).

Listeriosis is a relatively rare food-borne illness, but can be life threatening with high fatality rates. It is mainly associated with the consumption of processed foods that require no further cooking by the consumer (Schlech, 2000). Contamination of RTE poultry products can occur after cooking by cross-contamination environmentally or via workers, surfaces and equipments (Osaili *et al*., 2011). *Staph.aureus* produces staphylococcal enterotoxins (SEs) in contaminated food and symptoms of staphylococcal food intoxication generally occur within one to six hours after the ingestion of food and the common symptoms are nausea, vomiting, abdominal cramps, and diarrhea (Adwan *et al*., 2005).

Nowadays, *Salmonella* is one of the most pathogenic genera implicated in food-borne bacterial outbreaks and diseases and constitute an important public health problem. There are numerous transmission routes for Salmonellosis, but the majority of the human infections are derived from consumption of contaminated poultry products (*Saha et al*., 2016). Thermo-tolerant bacteria belonging to Campylobacter genus (especially *C. jejuni*, *C. coli*, *C. lari* and marginally *C. upsaliensis*) are recognized as leading human foodborne pathogens causing an acute gastrointestinal disease called campylobacteriosis **(**Vondrakova *et al*., 2014).Chicken meat must be of a high microbiological quality in order to ensure that the consumer receives a product that is not spoilt, or does not carry foodborne disease.

RTE ccooked chicken meat can be consumed without further cooking. Therefore, the presence of pathogens in these products could present a food safety threat. So, the aim of the present study was to determine the prevalence of *L. monocytogenes*, *Staph. aureus*, *Salmonella* spp. and *Campylobacter* spp. in cooked chicken meat marketed in different restaurants in Assiut city, Egypt as well as discuss the public health importance of the isolated organisms.

II. Materials And Methods

Sample collection

A total of 135 samples of RTE cooked chicken meat comprised of 50 samples of RTE chicken frankfurters, 35 RTE chicken shawerma, 25 cooked chicken breast and 25 cooked chicken thigh. These RTE chicken samples were purchased and transferred to the laboratory in sterile and sealed plastic containers.

Sample preparation

At the laboratory, each sample was aseptically and carefully freed from its plastic bag and cut into fine particles by sterile scissor, then homogenized and thoroughly mixed. *Listeria* spp. was isolated from cooked chicken meat and its products through methods carried out by Hudson, *et al*. (1994). The suspect Listeria colonies were further confirmed according to ISO 11290–1 (1996). Isolation of *S. aureus* was performed according to Singh and Prakash (2008). Further detection of *S. aureus* enterotoxins was carried out by ELISA (Ewalid, 1988). *Salmonella* isolation conducted as method described by ISO-6579 (2002). Serological identification of Salmonellae was carried out according to Kauffman–White scheme (Kauffman, 1974) for the determination of Somatic (O) and flagellar (H) antigens using Salmonella antiserum (DENKA SEIKEN Co., Japan). Isolation of *Campylobacter* spp. performed according to Bolton *et al*. (1984) and FDA (1998). Serological identification of *Campylobacter* spp. was done by Latex Agglutination Kit species according to (Oyarzabal *et al*., 2007).

III. Results And Discussion

Table 1. Prevalence of food borne pathogens in the examined samples of cooked chicken meat and products

Samples	No.	*L*isteria spp.		*Staph. aureus*		*Salmonella* spp.		*Campylobacter* spp.	
		No.	%	No.	%	No.	%	No.	%
Frankfurter	50	3	6	12	24	0	0	0	0
Shawerma	35	1	2.9	3	8.6	0	0	2	5.7
Breast	25	11	44	1	4	2	8	0	0
Thigh	25	7	28	6	24	4	16	1	4
Total	135	9	6.7	22	16.3	6	4.4	3	2.2

Table 2. Incidence of different types of *Listeria* spp. in the examined RTE cooked chicken meat and products

Samples	No.	*L. monocytogenes*		*L. innocua*		*L. welshimeri*		*L. grayi*		*L. ivanovii*	
		+ve	%	+ve	%	+ve	%	+ve	%	+ve	%
Frankfurter	50	3	6	0	0	0	0	0	0	0	0
Shawerma	35	1	2.9	0	0	0	0	0	0	0	0
Breast	25	6	24	3	12	0	0	2	8	0	0
Thigh	25	3	12	3	12	0	0	1	4	0	0
Total	135	9	6.7	6	4.4	0	0	3	2.2	0	0

Table 3. Incidence of Coagulase positive *Staph. aureus* in the samples of cooked chicken meat and products

Samples	No.	Coagulase positive /DNAase positive		Enterotoxin production		Types of toxin
		No.	%	No.	%	
Frankfurter	50	12	24	-	-	----
Shawerma	35	3	8.6	1	2.9	A and C
Breast	25	1	4	-	-	----
Thigh	25	6	24	-	-	----
Total	135	22	16.3	1	0.7	

Table 4. *Salmonella* serovars from the examined samples of cooked chicken meat and products

Samples	No.	*S.* Enteritidis		*S.*Typhimurium		*S.* Kentucky		*S.* Infantis	
		No.	%	No.	%	No.	%	No.	%
Frankfurter	50	0	0	0	0	0	0	0	0
Shawerma	35	0	0	0	0	0	0	0	0
Breast	25	2	8	0	0	0	0	0	0
Thigh	25	1	4	1	4	1	4	1	4
Total	135	3	2.2	1	0.7	1	0.7	1	0.7

Table 5. Incidence of *Campylobacter* spp. in the samples of cooked chicken meat and products

Samples	No.	*C. jejuni*		*C. coli*		*C. lari*	
		No.	%	No.	%	No.	%
Frankfurter	50	0	0	0	0	0	0
Shawerma	35	2	5.7	0	0	0	0
Breast	25	0	0	0	0	0	0
Thigh	25	1	4	0	0	0	0
Total	135	3	2.2	0	0	0	0

Safety of RTE cooked chicken meat and products depend on proper cooking and post processing sanitary practices. Recontamination of such meat during post-processing may be the cause of outbreaks of food-borne disease (Ansari, 2015). The presence of pathogens on surfaces of equipment or the environment, particularly in post-cooking areas, serves as one of the most important routes for contamination of RTE meats (Syne *et al*., 2013). Regarding RTE chicken frankfurters, the findings outlined in Tables 1 and 2 showed that *Listeria* spp. were isolated from 3 out of 50 examined samples of RTE chicken frankfurters with an incidence of 6%; all identified as *L. monocytogenes*. In contrast, higher prevalence rate (0-16%) of *L. monocytogenes* contamination found in frankfurter packages that produced in various commercial processing plants was recorded by Wallace *et al*. (2003).

Six species comprise the genus Listeria (ICMSF, 1996). *L. grayi* and *L. innocua* are considered non-pathogenic, while *L. seeligeri*, *L. ivanovii*, and *L. welshimeri* are rarely causes of human infection. *L. monocytogenes* is the most important species with respect to human health. RTE meats are especially a concern since these may be consumed without further cooking and are known to be good growth substrates for pathogenic microorganisms such as *L. monocytogenes* (Zhu, *et al*. 2005). Moreover, *L. monocytogenes* is an occasional post process contaminant of RTE meats such as frankfurters and is responsible for foodborne illness outbreaks and recalls of the subsequently adulterated food products (Sommers *et al*., 2010). Thus, RTE meats contamination by *L. monocytogenes* presents a greater public health threat because these prepared foods can be contaminated during post-cook handling (Prencipe *et al*., 2012). Out of 50 examined RTE chicken frankfurters in the present study, 12 samples with an incidence of 24% were contaminated with coagulase positive *Staph. aureus* (Tables 1 and 3). On the contrary, lower incidence (7.5%) of coagulase positive *Staph. aureus* contamination in examined chicken frankfurters was recorded by Elbagory *et al*. (2005). The higher contamination rate with *Staph. aureus* in this study may be attributed to excessive handling of chicken meat and its products by workers during processing, bad personal hygiene, cross contamination, and slicing machines or cutting knives (Waldroup, 1996).

Staphylococcal food poisoning (SFP) is one of the most common food-borne diseases that affects hundreds of thousands of people each year worldwide (Ji-Yeon *et al*., 2013). Hosts of *Staph. aureus* are food handlers especially at nasal areas and hands which is the vehicle of enterotoxigenic *Staph. aureus*. Furthermore, food handlers are important source of staphylococcal food contamination in restaurants and food outlets (Colombari *et al*., 2007). The presence of *Staph. aureus* or its enterotoxins in processed food is generally an indication of poor sanitation (Reginald and Gayle, 2001).

Results in the current study indicated that *Salmonella* spp. couldn't be detected in chicken frankfurters (Table 1). Absence of Salmonellae in heat-treated poultry meat products (such as frankfurter) may be attributed to the thermal treatment used in processing procedures was effective for killing these sensitive bacteria (Murphy *et al*., 2004). In addition, spices which include parsley, black pepper, garlic, nutmeg, and mustard, among others are used in chicken frankfurters to impart flavor and enhance aroma of the finished product; these spices are added for their antimicrobial effects (Brown, 2009). On the other hand, Saad *et al*. (2015) reported that *Salmonella* spp. were isolated from 15% of the examined samples of fully cooked chicken frankfurters.

Data presented in Table (1) showed that *Campylobacter* spp. couldn't detect from 50 examined RTE chicken frankfurters. This result is in line with most of the findings involving cooked chicken products (Nur Ilida, and Faridah, 2012). Also, a study done by Joint Food Safety and Standard Group of United Kingdom (1996) found that 758 cooked chicken products analyzed were free from Campylobacter. Heating at 55°C and above, rapidly inactivates the organism. Thorough cooking kills *Campylobacter* spp. as indicated by Rob *et al*. (2003). Furthermore, all frankfurters are formulated with lean meat and fat as main ingredients. Other non meat ingredients include salt, polyphosphates, binders (milk powder, corn starch), ice, spices and nitrite (Feiner, 2006) thus; failure of isolating *Campylobacter* spp. could be due to a high concentration of salt and other ingredients. Chicken shawerma is one of the most RTE sandwiches sold in fast food restaurants in Egypt. It is a wrap of shredded chicken meat prepared by alternately stacking strips of fat and pieces of seasoned meat on a rotating vertical skewer. The meat is roasted from the outside, while most of the inside remains rare. Shavings are cut off the block of meat for serving, and the remaining block of meat is kept heated on the rotating skewer (Essa *et al*., 2007; Banna-Hanin and Nawas, 2016). Fat and salt content of shawarma was ranged between 20 and 40 g/100 g and 1–2 g/100 g, respectively and the weight of shawerma was usually between 10 and 15 kg (Vazgecer *et al*. 2003). In this research, *L. monocytogenes* was determined in 1 (2.9%) samples of RTE chicken shawerma (Tables 1 and 2). Comparatively, Alsheikh *et al*. (2013) recorded slightly higher isolation rate (4%) of *L. monocytogenes* in chicken shawerma samples purchased from restaurants in Khartoum state Sudan. Also, *L. monocytogenes* was isolated from 12 (4%) of 301 chicken shawerma samples in Jordan (Osaili *et al*., 2014). On the other hand, higher records were reported by several investigators as Moustafa El-Shenawy *et al*. (2011) who found *L. monocytogenes* in 3 (12.5%) of 24 samples of street-vended RTE shawerma in Alexandria city. Moreover, In Amman, Jordan, Osaili *et al*. (2011) isolated *L.monocytogenes* from shawerma with percent 13.3% of samples. The contamination by *L. monocytogenes* in RTE meat primarily occurs during slicing and

packaging after cooking. In addition, cross-contamination between raw materials, equipments, utensils, humans could contribute to the spread of *L. monocytogenes* in food processing plants (Jemmi and Stephen, 2006). The findings outlined in Tables 1 and 3 showed that *staph aureus* were isolated from 3 of 35 examined chicken shawerma with incidence of 8.6%. Furthermore, enterotoxigenic *S. aureus* was detected in 1 (2.9%). The enterotoxins produced were identified as staphylococcal enterotoxin A (SEA) and staphylococcal enterotoxin C (SEC). These result was quite agree with Nimri-Laila *et al*. (2014) who succeeded in isolation of this pathogen with percentage of 8.3% in Jordan.

In the present study, *Staph*. *aureus* isolated from shawerma proved to be enterotoxin producers **(**SET A and C), while the strains isolated from chicken frankfurter, cooked breast and cooked thigh were failed to produce enterotoxins **.**On the other hand, at Al-Taif Governorate, KSA, Sharaf-Eman and Sabra-Sherifa (2012) couldn't detect any staphylococcal contamination in the examined RTE chicken shawerma. While, higher incidence of coagulase positive *Staph. aureus* contamination in examined RTE chicken shawerma were recorded by Ali-Sohaila and Abd-Elaziz-Doaa, (2011) (30%); El-Dosoky (2013) (15%) and Hassan *et al*. (2016) (22.8%). The presence of *Staph. aureus* in RTE chicken meat may be attributed to inadequate cooking, improper holding temperatures, or poor personal and equipment cleanliness (Shafizi *et al*. 2016). Furthermore, fully cooked foods support the opportunity for growth of coagulase-positive staphylococci and toxin production that is because the absence of competing microorganisms. So, coagulase-positive staphylococci appear to be of greatest concern in products such as RTE chicken meat (Singh, 2017).

Shafizi *et al*., (2016) recorded that the incidence of *Staph*. *aureus* was 2.3% for fully cooked food for immediate sale or consumption. They declared that these results could be due to inadequate cooking, improper holding temperatures, or poor personal and equipment cleanliness. The obtained results showed that no Salmonella could be recovered in RTE chicken shawerma in this study (Tables 1 and 4). This achieved result quite agreed with Amin (2015) who couldn't detect *Salmonella* in RTE chicken shawerma. This may be due to chicken shawarma contain sliced chicken seasoned with peppers and with a special garlic spread. Garlic is widely known for its antibacterial properties with a notable effect on *Salmonella* spp. and will reduce any bacterial count to a dose that is harmless (Banna-Hanin and Nawas, 2016).

In contrast, higher prevalence rate of *Salmonella* spp. in examined chicken shawerma was recorded by Nimiri-Laila *et al*. (2014) (25.5%); Ahmed- Amany *et al*. (2015) (15%) and (Banna-Hanin and Nawas, 2016) (30%). Moreover, Hassanin *et al*. (2014) found that 13.3% of the examined chicken shawerma samples were positive for *S*. Enteritidis. The results obtained in Tables (1) and (5) illustrated that out of a total of 35 chicken shawerma samples screened for isolation of *Campylobacter* spp., 2 (5.7%) were positive for these organisms. The two strains classified as *C jejuni*. On contrast, the higher incidence (19%) was recorded by El Fadaly *et al*., (2016) in RTE chicken shawerma collected from different restaurants in Assiut governorate. While, Nimri-Lailia *et al*. (2014) couldn't detect *Campylobacter* spp. from shawerma samples. Moreover, previous studies found that contamination by *C. jejuni* can be transferred from the cutting boards and kitchen cutlery from raw chicken to cooked chicken (Tang *et al*., 2011). Chicken shawarma are manipulated extensively during processing and therefore have a potential for high bacterial contamination levels on the surface and the depth of the meat. As a result, there is an increased risk of pathogens surviving and transferring not only by cross-contamination, but also by undercooking as observed in this kind of fast-food industry (Nimri-Laila *et al*., 2014).

With regard to RTE cooked chicken breast, it is evident from the data presented in Tables 1 and 2 that 11 samples with an incidence of 44% were contaminated with *Listeria* spp. and distributed as follows: *L. monocytogenes* (24 %), *L. innocua* (12 %) and *L.grayi* (8 %). On the contrary, in a related study performed by Diaz-Lopez *et al*. (2011), the presence of *L. monocytogenes* from grilled chicken was not detected by culture or PCR.

The results presented in Table (1) demonstrated that 1 (4%) coagulase positive *Staph. aureus* strains which were isolated from the examined chicken breast samples were analyzed for their toxigenic capabilities by using ELISA technique. All samples were free from enterotoxigenic. In contrast, higher prevalence rate (17%) of *Staph. aureus* from RTE grilled chicken meat was recorded by Manguiat and Fang (2013). On the other hand, the results obtained in this study disagreed with Tavakoli and Riazipour (2008) who couldn't find *Staph. aureus* in grilled chicken in Tehran Universities Restaurants. Manguiat and Fang (2013) explained that contamination in grilled chickens may come from uncooked and contaminated ingredients, such as condiments and spices (e.g., pepper and chili) that usually were added after grilling. While sufficient grilling destroys the bacteria, cross contamination by these ingredients is very likely.

The findings outlined in Tables 1 and 4 showed that *Salmonella* spp. were isolated from 2 out of 25 examined RTE breast samples with an incidence of 8 %. The two isolated serovars were identified as *S.* Enteritidis with a percentage of 8 %. This obtained result agreed to some extent with Kozačinski *et al*. (2006) who detected *Salmonella* by percentages of 9.52% of chicken breasts. In contrast, higher prevalence rate (13.3%) of *S*. Enteritidis was recorded by Hassanin- Faten *et al*. (2014) in RTE chicken pane. On the other hand,

the results obtained in the present study disagreed with Tavakoli and Riazipour (2008) who couldn't detect *Salmonella* spp. from the examined RTE grilled chicken in Tehran Universities Restaurants. In general, presence of Salmonella in RTE food is a great risk to consumer health, there is zero tolerance towards its presence in RTE products (Akbar and Anal, 2015).

The results outlined in Table 1 showed that *Campylobacter* spp. couldn't isolated from 25 examined RTE chicken breast. Comparatively, this obtained result was in agreement with Diaz-Lopez *et al*. (2011) who couldn't detect *C. jejuni* by PCR in grilled chicken in Reynosa city, Mexico. In contrast, higher prevalence rate (16.6%) of *Campylobacter* spp. was recorded by El Fadaly *et al*. (2016) in RTE grilled chicken tissues (core portions).

Our data obtained in the current study showed that 7 (28 %) out of 25 RTE chicken thigh samples were contaminated with *Listeria* spp. (Table 1). The identified *Listeria* spp. were *L. monocytogenes* (12 %), *L. innocua* (12 %) and *L.grayi* (4 %) as demonstrated in Table (2). The presence of *L. monocytogenes* in cooked chicken meats could be explained by the inadequate heat treatment to destroy the growth of *L. monocytogenes* or as a result of post process contamination (Alsheikh *et al*., 2013).

The data outlined in Table (1) illustrated that out of 25 samples of cooked chicken thigh examined in the present study, 6 samples with an incidence of 24% were contaminated with coagulase positive *Staph. aureus*. On the contrary, lower incidence (9.3%) of *Staph. aureus* contamination of RTE grilled chicken samples from street vendors in Mexico, was recorded by Diaz-Lopes *et al*. (2011). Also, the results obtained in the present study disagreed with Senait and Moorty (2016) who reported that *Staph. aureus* couldn't isolated from any of the examined roasted chicken samples in Ethiopia. The presence of these pathogens is of primary concern because some strains are capable of producing a heat-stable enterotoxin that causes food poisoning in humans, and should therefore be taken into account in risk assessment (Nimri-Laila *et al*., 2014).

Out of 25 examined RTE chicken thighs in the present study as shown in Tables (1) and (4), 4 samples with an incidence of 16% were contaminated with *Salmonella* spp. One sample was identified as *S.* Typhimurium (4%), the another sample was classified as *S.* Enteritidis (4%) and one was *S.* Infantis (4%) while, the last sample were categorized as *S.* Kentucky (4%). Nearly similar isolation rate (16.7%) of *Salmonella* spp. from RTE grilled chicken was obtained by Manguiat and Fang (2013). In a related study, lower incidence (0.8%) of *Salmonella* spp. in RTE chicken products was obtained by Osaili *et al*. (2014). Diaz-Lopez *et al.* (2011) couldn't isolate *Salmonella* spp. by using PCR from RTE grilled chicken from street vendors and retail outlets in the city of Reynosa, Mexico. On the contrary, higher prevalence rate (25.71%) of *Salmonella* spp. was recorded in chicken thigh meat samples by Ruban and Fairoze (2011). Also, higher incidence (7%) of *S.* Kentucky in the chicken thigh was recorded by Ibrahim-Hemmat *et al*. (2014). The results of this study indicated that *S.* Enteritidis was the most isolated serovar from cooked chicken. These results quite agreed with Banna-Hanin and Nawas (2016) who emphasized that *S.* Enteritidis is the most common serotype of Salmonella contaminating food. The increased prevalence in poultry products made *S.* Enteritidis a food-safety issue as of the 1970s. The detection of *Salmonella* spp. in a 25-g sample is considered potentially hazardous or unacceptable according to the microbiological guide that was used. Furthermore, the presence of *Salmonella* has been associated with inadequate cooking, cross-contamination from an unhygienic environment, and food handlers (Manguiat and Fang, 2013). Out of 25 examined chicken thigh in the present study as shown in Tables 1 and 5, one sample with an incidence of 4% was contaminated with *C. jejeni*. In this study, contamination of RTE cooked chicken thighs with *C. jeujeni* probably because such cuts are most at risk in view of the anatomical proximity to the final part of the digestive tract (Mezher *et al*. 2016). In Mexico City, a survey of RTE roasted chickens showed that such product was contaminated with *C. jejuni* by percent 41% (Quinones-Ramirez *et al*., 2000).

IV. Conclusion

This study indicated that RTE chicken meats are often contaminated with *Staph. aureus*, *L. monocytogenes*, *S.* Enteritidis, *S.*Typhimurium and *C. jejuni*. Chicken are potential vehicles for transmitting food-borne diseases, thus represent a potential threat to consumer health. As RTE chicken meat are of great demand because of its easy use and time saving, but not subjected to further heating before consumption so, these results signify the importance of sustained surveillance of foodborne pathogens in cooked chicken meat to minimize the risk of contamination during production, cooking, and serving and to assure the microbiological safety of RTE food to safeguard public health. There is need for the relevant local authorities to ensure that the food sold to consumers in fast food restaurants is safe, wholesome and fit for human consumption in order to prevent outbreaks of food-borne illnesses. Also, there should be regular training/retraining and health education of these food handlers in all aspects of food hygiene and safety. In addition, ensuring good quality raw materials, adequate lethality treatment, and effective sanitation of both the equipment and processing environment are crucial in preventing contamination of RTE cooked chicken meats.

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