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Prevalence and Molecular Characterization of *Salmonella* spp. in Poultry Meat and Its Products

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ABSTRACT

One hundred random samples of poultry meat and its products, 50 of which were fresh cuts-up (chicken breast and chicken thigh) and 50 of which were frozen half-cooked products of various brands (chicken burger and chicken nuggets), 25 of each, collected from retailers and poultry butcher shops at Menoufia governorate, Egypt. Results revealed that the prevalence of *Salmonella* spp. was 6% in the examined poultry meat samples. The prevalence was as the following: chicken breast 8% (2 out of 25 samples), chicken thigh was 8% (2 out of 25 samples), chicken nuggets was 4% (1 out of 25 samples), and chicken burger was 4% (1 out of 25 samples). The highest prevalence of *Salmonella* spp. was detected in chicken thigh and chicken breast while the lowest was in both chicken nuggets and chicken burger. *Salmonella* was typed by using antisera and resulted in 3 different serotypes, one isolate was *Salmonella* paratyphi A, one isolate was *Salmonella* Heidelberg, and four isolates were *Salmonella* Typhimurium. The highest rate of isolation was *Salmonella typhimurium* (4%). R-T PCR confirmed that all the isolates were *Salmonella* spp. by targeting *invA* gene specific for *Salmonella* spp.

Key words: *invA* gene, Poultry meat, R-T PCR and *Salmonella* spp.

INTRODUCTION

The poultry sector has seen remarkable growth in recent years, which may be related to the fact that chicken production has affordable costs, is very nutritious, grows quickly, and yields exceptional forms of further processed products (Barbut, 2015). Egypt currently has a sizable consumer base that consumes chicken and is self-sufficient. One of the most common animal protein sources among Egyptians is chicken flesh. Additionally, burgers and other processed chicken-based items have also been widely sold through restaurants and retailers and consumed by the public (Moubasher et al., 2016). One of the biggest challenges confronting the worldwide food industry is the presence of harmful microorganisms, mainly bacteria, in chicken products, including uncooked broiler meat, which

has become a significant source of contamination (Pesewu et al., 2018). Bacterial contamination occurs at any stage of the production process, from the farm where the food is initially grown, all the way to when it's consumed by the customer. This includes contamination that may occur during primary production on the farm, during transportation of live poultry, during slaughtering processes, in the environment of the slaughterhouse, and even during storage until it reaches the consumer (Ananchaipattana et al., 2012). Every year, approximately 76 million people in the United States suffer from foodborne illnesses, resulting in 325.000 hospitalizations and 6.000 deaths. Salmonellosis is the most frequently reported zoonotic disease in humans, with 131.468 confirmed cases in the US (EFSA, 2010). In numerous developing countries, such as Brazil, India, Egypt, and Zimbabwe, instances of salmonellosis have historically been most linked to poultry products (Yang et al., 2011). *Salmonella* infection is a potential hazard at each stage of processing because the leftover bacterium is introduced to the poultry production system when live birds are processed. *Salmonella* may therefore spread from carcass to carcass during the manufacturing steps (Nidaullah et al., 2017). *Salmonella* contamination in food can cause non-typhoidal salmonellosis, one of the world's most common causes of gastroenteritis and deaths associated with it (Rajan et al., 2017). Therefore, the present study was designed for: collection of random samples of poultry meat and its products from supermarkets and poultry butcher shops, isolation of *Salmonella* spp. by conventional standard methods, biochemical confirmation of the suspected isolates, serotyping of the isolated strains, and RT-PCR for detection of *Salmonella*.

MATERIALS AND METHODS

Collection of samples:

One hundred randomly selected samples of poultry meat and its products. The samples were divided into two categories: 50 fresh cuts (chicken breast and chicken thigh) and 50 frozen, breaded, half-cooked products from various brands (chicken burger and chicken nuggets), 25 of each. Samples were collected from poultry butcher shops and retailers in Menoufia governorate, Egypt between May 2021 to June 2022. To prevent contamination, each sample was placed in a separate sterile plastic bag, kept in an icebox at a temperature of 0-4 ^{o}C, and transported to Food hygiene and control research lab, Faculty of Veterinary medicine, University of Sadat City, Egypt under aseptic conditions (Ethical approval number: VUSC-008-1-23).

Preparation and Conventional bacteriological examination (ISO 6579:2002):

The Samples (25 gm) were pre-enriched in non-selective liquid media (buffered peptone water (BPW) 1.0%) (Oxoid), homogenized in the stomacher machine for a minimum of 2.5 minutes and then incubated at 37 ± 1 °C for 18± 2 hrs. 1.0 ml of each pre-enrichment was used to inoculate 10 mL of Muller Kauffman tetra thionate-novobiocin broth base (MKTTn) (Oxoid) which was incubated at 37 ± 1°C for 24 ± 3 hrs and 0.1 ml of each pre-enrichment was used to inoculate 10 ml of Rappaport-Vassiliadis with soya broth (RVs) (Oxoid) and incubated at 41.5 ± 1 °C for 24 ± 3 hrs. After selective enrichment a loopful of the RVs broth and MKTTn broth was streaked on two agars in parallel one of them was xylose lysine desoxycholate agar media (XLD) (Oxoid) and the second was Bismuth sulphite agar media (BS) (HiMedia) then plates were incubated in 37 ± 1 °C for 24 ± 3 hrs.

Identification (ISO 6579:2002):

The suspected *Salmonella* colonies were subcultured on the same media of purification and then the pure culture was examined morphologically (films were stained with Gram stain which showed pink to red Gram-negative bacteria), then biochemically examined.

Biochemical confirmation (ISO 6579:2002):

Serotyping (Kauffman, 1974):

To identify the specific type of *Salmonella*, the Kauffman-White scheme was used. This involved testing for somatic (O) and flagellar (H) antigens using *Salmonella* antiserum from DENKA SEIKEN Co. in Japan. To prepare for testing, a small amount of the colony being studied was mixed with a drop of sterilized normal saline solution on a clean glass slide using a loop. This was done to create a turbid suspension. The slide was then gently moved back and forth for 30-60 seconds, and the results were observed against a dark background, preferably with the assistance of a magnifying glass.

Examination for somatic (O-antigen) "slide agglutination test": A dense suspension of an organism was created by suspending it in 0.5 ml of saline solution. A microscopic slide was marked with two circles of around 1 cm in diameter using a wax pencil. One of the marked circles was used to put a drop of *Salmonella* Polyvalent "O" antiserum, while the other circle was used for the negative control, which received a drop of saline solution. Using a clean dropper, a drop of bacterial suspension (0.05 ml) was added to each of the circles and mixed thoroughly by gently racking for 1-2 minutes to avoid excessive evaporation. A positive reaction was characterized by rapid and complete agglutination, while a delayed or partial agglutination was considered negative. To identify the *Salmonella* group and other somatic components of the group, separate "O" antiserum factors were used.

Examination for flagellar (H) antigen "tube agglutination test": The complete antigenic formula of the isolates was determined by using Polyvalent H antiserum for both phase 1 and phase 2 to determine the flagellar (H) antigens. In the first phase, the same procedure was followed, but only one drop of the anti H serum was used. In the second phase, a pure non-agglutinable colony was inoculated on a semi-solid nutrient agar and incubated for 24 ± 3 hours at 37 ± 1 ºC, and the H-antigens were examined using the culture, following the same procedure as in the first phase if needed.

Molecular characterization (R-T PCR):

DNA was extracted using QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). The list of primers and probes used in the study is provided in Table 1 and Table 2. Quanti Tect probe RT-PCR kit handbook. (January 2008) was used in the R-T PCR. A 25.175 µL reaction was prepared to contain 12.5 µL PCR Master Mix, 0.5 µL (50PMOL) of each primer, 0.125µl (30 PMOL) of probe, 4.55 µlRNase Free Water, and 7 µl of template DNA. The following thermal profile was used (Cheng et al., 2008): initial denaturation at 95 ˚C for 3 min; 50 cycles each consisting of denaturation at 95˚C for 30 s, annealing at 60˚C for 30 s and extension at 60˚C for 30 s; and final extension at 72 ˚C for 10 min. Real time PCR machine was adjusted for detection of *Salmonella* spp. Once all tubes had been prepared, the real time PCR amplification was performed. The sample was considered positive if it has a CT value or crossing point less than 35.0 cycles with a typical amplification curve and was considered negative if it has no

CT value (or crossing point) and no amplification curve.

Table (1): Oligonucleotides primer sequence used in R-T PCR for amplification of *invA* gene specific for *Salmonella* spp:

Targeted genus	**Targeted gene**	**Primer pair name**	**Primer sequence (5'- 3')**	**Size (bp)**	**Reference**
Salmonella spp.	*Salmonella* specific invasion protein (*invA*) gene	*invA*3F	AACGTGTTTC CGTGCGTAAT	262	**Cheng et al. (2008)**
		*InvA*3R	TCCATCAAAT TAGCGGAGGC		

Table (2): Oligonucleotides probe sequence used in R-T PCR for amplification of *invA* gene specific for *Salmonella* spp.

Targeted genus	**Targeted gene**	**TaqMan probe name**	**Probe sequence (5'- 3')**	**Reference**
Salmonella spp.	*Salmonella* specific invasion protein (*invA*) gene	*invA*Probe1	FAM-TGGAAGCGCTCGCATTGTGG-BHQ-1	**Cheng et al. (2008)**

RESULTS & DISCUSSION

Results of conventional bacteriological examination:

Table (3): Prevalence of *Salmonella* spp. in the examined samples of poultry meat and its products (n = 25 OF EACH):

Samples	**No. of examined samples**	**Positive samples**	
		NO.	**(%)**
Chicken breast	25	2	8
Chicken thigh	25	2	8
Chicken nuggets	25	1	4
Chicken burger	25	1	4

Table (4): No. and prevalence of *Salmonella* spp. serotypes in the examined samples (n = 25 OF EACH):

Samples / Isolated serotypes	Chicken breast		Chicken thigh		Chicken nuggets		Chicken burger		Total (100)	
	No	**%**	**No**	**%**	**No**	**%**	**No**	**%**	**No**	**%**
S. heidelberg	-	-	-	-	-	-	1	4	1	1
S. paratyphi A	-	-	1	4	-	-	-	-	1	1
S. tyhimurium	2	8	1	4	1	4	-	-	4	4

Table (5): Antigenic formula of different *Salmonella* isolates:

Serotypes	Group	Somatic (O) antigen	Flagellar (H) antigen	
			Phase (1)	**Phase (2)**
S. heidelberg	B	1, 4, [5], 12	r	1, 2
S. paratyphi A	A	1, 2, 12	a	[1, 5]
S. typhimurium	B	1, 4, [5]	i	1, 2

Table (6): Acceptability of the examined samples with *Salmonella* spp. according to Egyptian standards (2005):

Samples	Microorganism	Accepted		Unaccepted		ES (2005)
		No.	**%**	**No.**	**%**	**PL**
Chicken breast	*Salmonella* spp.	23	92	2	8	**Free in 25 gm meat**
Chicken thigh	*Salmonella* spp.	23	92	2	8	
Chicken nuggets	*Salmonella* spp.	24	96	1	4	
Chicken burger	*Salmonella* spp.	24	96	1	4	

Egyptian Standards (2005):

ES: 1651/2005 for chilled poultry and rabbit meat "chicken breast and chicken thigh".

ES: 3493/2005 for heat treated poultry products "chicken nuggets".

ES: 2910/2005 for poultry and turkey meat products "chicken burger".

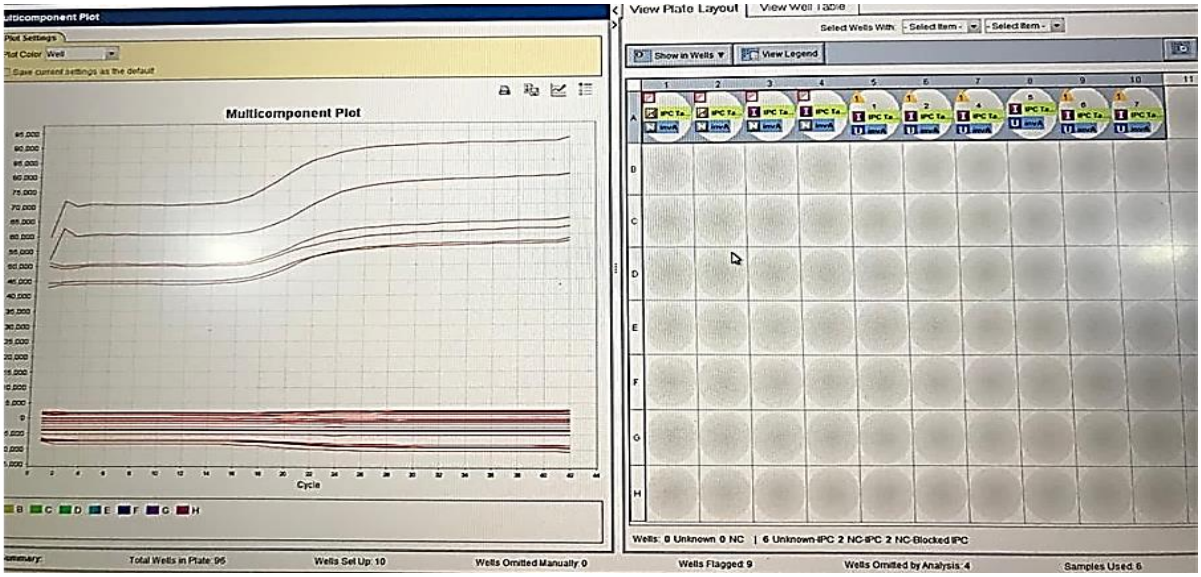

Figure (1): Representative positive results by R-T PCR:

Salmonella contamination of poultry processing plants continues to be a problem worldwide, especially as demand and output for poultry increases. Public health is still seriously affected by *Salmonella*-related food outbreaks. *Salmonella* can survive well in poultry meat, so it is essential for poultry processing companies to continuously improve their processes to decrease the hazard of contamination of their final products with *Salmonella*. During the present work, a total of 6% (6 out of 100 samples) of poultry meat and its products were positive for *Salmonella* spp. Relating to the results recorded in table (3); it is evident that the prevalence of *Salmonella* spp. in the examined samples was as follow; chicken breast (8%), chicken thigh (8%), chicken nuggets (4%), and chicken burger (4%), respectively. Consequently, the highest prevalence of *Salmonella* spp. was detected in chicken thigh and chicken breast (the same percentage), whereas chicken nuggets and chicken burger had the lowest prevalence (the same percentage). This could be attributed to workers' hands, exposing the thigh and breast samples to fecal contamination during evisceration, while low results were detected in chicken burger and chicken nuggets samples due to freezing of these products which may affect on the viability of *Salmonella*. Nearly analogous results were obtained by Abdel-Aziz (2016) who found that the overall prevalence of *Salmonella* contamination of 75 examined samples was (6.6%)., and Akbar and Anal (2013) who tested a total number of 209 poultry meat samples and out of which,5.26% were found to be contaminated with *Salmonella*. On the contrary, higher results were obtained by Harb et al. (2018) who detected *Salmonella* spp. with a prevalence of 16% from the examined 100 frozen chicken samples., and El Shrek and Ali (2012) who inspected uncooked chicken burger samples (n = 56) and detected *Salmonella* spp. in 12.5% of the examined samples. Whereas lower results were obtained by Oueslati et al. (2022) who detected that the prevalence of *Salmonella* contamination in breast muscle was estimated to be (0.9%)., and Rabie et

al. (2012) who examined 50raw frozen chickens meat and the incidence of *Salmonella* was 2 (4%). Although Osaili et al. (2014) failed to detect *Salmonella* spp. in 20 chicken burger samples and Karmi (2014) wasn't able to isolate *Salmonella* in 10 chicken luncheon and 10chicken burger samples. The higher or lower results compared to the current work may be due to the variance in hygienic conditions during slaughtering, evisceration, processing, and post processing.

CONCLUSION

The obtained data in this work allow concluding that some of the examined samples were contaminated by *Salmonella* spp. (6%) which constitutes a public health hazard to the consumers. This suggests that sanitary practices were neglected during the production, handling, processing, and post processing of the examined samples or predisposing factors that contributed to the bacterial growth, such as high moisture content, product sweating, and poor product storage, or may be because that the poultry had salmonellosis while being alive and not be treated, so the bacteria are still present after slaughtering.

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