Incidence of Salmonella species in chicken cut -up carcasses and chicken products

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A B S T R A C T

A total of 125 samples weight 1kg of frozen packaged raw chicken cut- up (fillet, thigh and drumstick) and frozen packaged uncooked breaded chicken products (pane and drumstick) 25 of each collected from different retail shops and supermarkets for different companies at El Menofia Governorate. All samples were examined bacteriologically, serologically and multiplex PCR for isolation and identification of Salmonella species. Salmonella organisms were isolated from frozen packaged raw chicken cut- up (fillet, thigh and drumstick) and frozen uncooked packaged breaded chicken products (pane and drumstick) with percentages of 8%, 24%, 48%, 32% and 16% (the percent was according to n=25) and 1.6%, 4.8%, 9.6%, 6.4% and 3.2% (the percent was according to n=125), respectively. Moreover, the isolated Salmonella could be serologically identified as *S. enteritidis* was only detected in fillet by percent of 100% and *S. enteritidis*, *S. typhimurium* and *S. kentucky* were detected in drumstick by percent of 33% of each, but *S. enteritidis*, S. typhimurium and *S. anatum* were detected in thigh by percent of 33%, 50% and 17%, respectively. And also *S. enteritidis, S. typhimurium*, and *S. haifa* were detected in pane (uncooked breaded product) with percentages of 25%, 50% and 25%%, respectively. *S. typhimurium* and *S. Kentucky* were detected in drumstick (uncooked breaded product) with percentage of 50% of each. Also multiplex PCR methods were used for detection of virulence factors (invA, hil A, fimH and Stn genes) of *S. typhimurium* and *S. enteritidis* by PCR 2x Reddy Mix TM Master Mix (Thermo Scientific) with Cat No. AB0575/ LD-A, Waltham 02451, USA.

Key words: Salmonella spp., uncooked chicken cut-up, multiplex PCR, virulence factors.

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1. INTRODUCTION

In European Union (EU), Salmonella is the first notification cause of microbial foodborne contamination (Commission of the European Union, 2012). poultry meat and its products are very popular food throughout the world and no wonder since they are delicious, nutritious and considered as good and cheap source of protein characterized by good flavor and easily digested on the other hand, they rank first and 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). Several Salmonella specific virulence genes which takes an important role in the pathogenicity have been identified (Baumler et al. 2000). In Typhimurium serovar, at least 80 different virulence genes have been identified. Some genes are known to be Involved in adhesion and Invasion, like Sef (Clouthier et al., 1993), FimH (Duncan et al., 2005), InvA (Galan et al., 1992) and other genes associated with toxin production viz., Stn (Makino et al., 1999).

2. MATERIAL AND METHOD

2.1.Samples

A total of 125 samples weight 1 kg of frozen packaged raw chicken cut- up (fillet, thigh and drumstick) and frozen packaged uncooked breaded chicken products (pane and drumstick) 25 of each collected from different retail shops and supermarkets for different companies at El Menofia Governorate. The collected samples were taken under complete aseptic conditions and transferred, without undue delay, to the laboratory in an insulated ice box for microbiological examination.

2.2.preparation of samples:

The frozen samples were first thawed at below 5°C (for not longer than 12 hours). From each sample a quantity of 25g was taken using sterile tools (spoons, scalpels, knives and spatulas) (Zadernowska and Chajecka, 2012).

2.3. Microbiological detection of Salmonella:

Detection of Salmonella was done according to the ISO 6579 Standard-Microbiology of food and animal feeding stuff-Horizontal method for detection of Salmonella spp. (ISO 6579:2002+A1:2007) as follow:

2.3.1. Non-selective pre-enrichment broth:

Buffered peptone water was inoculated at ambient temperature with the test portion, and then incubated at 37 °C ± 1 °C for 18 h ± 2 h. The buffered peptone water was heated to 37 °C ± 1 °C before inoculation with the test portion.

2.3.2. Selective enrichment broth:

Rappaport-Vassiliadis Medium with soya (RVS broth) and Muller-Kauffmann Tetrathionate/novobiocin Broth (MKTTn broth) were inoculated with culture obtained from non-selective pre-enrichment. The RVS broth was incubated at 41.5 °C ± 1 °C for 24 h ± 3 h, and the MKTTn broth at 37 °C ± 1 °C for 24 h ± 3 h.

2.3.3. Selective Plating:

A loopful from the enriched broth was streaked onto the surface of previously prepared Xylose Lysine Desoxychoclate agar X.L.D. agar medium and was incubated at 37°C for 24 hours. Suspected colonies were red with or without black centers. The suspected colonies were sub-cultured onto nutrient agar plate and incubated at 37 °C ± 1 °C for 24 h ± 3 hours. Plates were examined for suspected Salmonella colonies which appear as red with or without black centers.

2.4. Biochemical confirmation tests:

Biochemical confirmation tests were done according to the standard ISO 6579:2002 as follow: Triple- sugar iron agar (TSI agar). Urease production (Christensen medium with urea). Peptone medium with tryptophan (indole reaction). Medium with Lysine (Lysine decarboxylation medium). Clark media (Voges-Proskauer (VP) reaction). ONPG medium (Reagent for detection of b-galactosidase)

2.5. Serological identification of Salmenolla:

Isolates proved biochemically to be Salmonella microorganisms were subjected to serological identification according to Kauffman white scheme (Kauffman, 1974) by using rapid diagnostic Salmonella antisera sets (Welcome Diagnostic, a Division of the Wellcome Foundation Limited, Dartford England DA15 AH) as follows: Isolates were sub cultured on nutrient slope for 24 hours at 37°C for application of slide agglutination technique, two homogenous suspensions were made on a slide by suspending a piece of suspected colony in a drop of sterile physiological saline. A drop of each of separate O and H Salmonella factors were added separately to each of the suspensions with standard loop thoroughly mixed to bring the microorganisms in close contact with antisera. Positive agglutination occurred within a minute and could be easily seen with the naked eye. A delayed

or partial agglutination was considered as negative or false result.

2.6. Detection of virulence of factors of S. typhymurium and S. enteritidis using Polymerase Chain Reaction (PCR):

PCR 2x Reddy Mix TM Master Mix (Thermo Scientific) with Cat No. AB0575/ LD-A, Waltham 02451, USA.

3. RESULTS

Results achieved in tables (1) (The incidence of Salmonella) were 8%, 24%, 48%, 32% and 16% (the percent was according to n=25) and 1.6%, 4.8%, 9.6%, 6.4% and 3.2% (the percent was according to n=125) of frozen packaged raw chicken cut- up (fillet, thigh and drumstick) and frozen uncooked packaged breaded chicken products (pane and drumstick), respectively. The incidence and serotyping of Salmonella isolated from frozen packaged raw chicken cut- up (fillet, thigh and drumstick) and frozen uncooked packaged breaded chicken products (pane and drumstick) were showed in table (2) as *S. enteritidis* was only detected in fillet by percent of 100% and *S. enteritidis*, *S. typhimurium* and *S. kentucky* were detected in drumstick by percent of 33% of each but *S. enteritidis*, *S. typhimurium* and *S. anatum* were detected in thigh by percent of 33%, 50% and 17%, respectively. And also *S. enteritidis*, *S. typhimurium*, and *S. haifa* were detected in pane (uncooked breaded product) by percent of 25%, 50% and 25%%, respectively. *S. typhimurium* and *S. kentucky* were detected in drumstick (uncooked breaded product) by percentage of 50% of each. The previous percentages were calculated according to only positive Salmonella samples. In the same table percentages were calculated by other ways such as *S. enteritidis* was 8% in fillet and *S. enteritidis*, *S. typhimurium* and *S. kentucky* were 8% drumstick but *S. enteritidis*, *S. typhimurium* and *S. anatum* were 16%, 24% and 8% in thigh, respectively. And also *S. enteritidis*, *S. typhimurium*, and *S. haifa* were 8%, 16% and 8% in pane (uncooked breaded product), respectively. *S. typhimurium* and *S. kentucky* were 8% of each in drumstick (uncooked breaded product). The previous percentages were calculated according to examine samples of each cut or product (n=25). At least the next percentages were calculated according to

Table (1): Incidence of Salmonella species in frozen packaged raw chicken cut-up (fillet, thigh and drumstick) and frozen uncooked packaged breaded chicken products (pane and drumstick). (n=25 for each).

Chicken meat Samples	No. of samples	Positive Salmonella	
		No.*	%** 25/125
A- Raw Chicken Cut- Up:			
I-Fillet	25	2	8/1.6
II-Drumstick	25	6	24/4.8
III-Thigh	25	12	48/9.6
B- Uncooked Breaded Chicken Products:			
I-Pane	25	8	32/6.4
II-Drumstick	25	4	16/3.2
Total No. of samples	125	32	25.6

No.* = Positive Salmonella. %** 25/125 = % Positive Salmonella in 25 samples /% Positive Salmonella in 125 total samples

Table (2): Incidence and serotyping of Salmonella isolated from frozen packaged raw chicken cut- up (fillet, thigh and drumstick) and frozen uncooked packaged breaded chicken products (pane and drumstick).

Samples	No. of examined samples	Positive Salmonella isolates		Salmonella serovars	Isolates			
		No.	%		No.	%*	%**	%***
A- Raw chicken cut-up:	25	2	8	*S. enteritidis*	2	100	8	6.25
I- Fillet								
II- Drumstick	25	6	24	*S. enteritidis*	2	33	8	6.25
				S. typhimurium	2	33	8	6.25
				S. kentucky	2	33	8	6.25
III- Thigh	25	12	48	*S. enteritidis*	4	33	16	12.5
				S. typhimurium	6	50	24	18.75
				S. anatum	2	17	8	6.25
B-Uncooked breaded chicken products	25	8	32	*S. enteritidis*	2	25	8	6.25
				S. typhimurium	4	50	16	12.5
I- Pane				*S. haifa*	2	25	8	6.25
II- Drumstick	25	4	16	*S. typhimurium*	2	50	8	6.25
				S. kentucky	2	50	8	6.25

% * = calculated from only positive Salmonella samples. % **= calculated from the examined samples (n=25). % *** = calculated from total positive samples (n=32).

total positive samples (n=32) as follow: *S. enteritidis* was 6.25% in fillet and *S. enteritidis*, *S. typhimurium* and *S. kentucky* were 6.25% in drumstick but *S. enteritidis*, *S. typhimurium* and *S. anatum* were 12.5%, 18.75% and 6.25% in thigh, respectively. And also *S. enteritidis*, *S. typhimurium*, and *S. haifa* were 6.25%, 12.5% and 6.25% in pane (uncooked breaded product), respectively. *S. typhimurium* and *S. kentucky* were 6.25% of each in drumstick (uncooked breaded product).

4. DISCUSSION:

The Salmonella contamination in chicken and chicken products had been widely investigated in many countries of the world but the prevalence varied (Soomro et al., 2010; Tibaijuka et al., 2003). The results of Hosam (1997) disagreed with the results in the current study as Salmonella failed to be detected from frozen packaged breast samples but in the current study Salmonella was 8%, Also the same author detected Salmonella in frozen packaged thigh at a percentage of 13.33% which is lower than those detected in the current study (48%) and the isolated serotypes were *S. enteritidis* and *S. typhimurium* but in the current study *S. enteritidis, S. typhimurium* and *S. anatum* 33%, 50% and 17% were detected, respectively. Moschonas et al. (2012) found that the surface-browned uncooked frozen breaded chicken products were associated with salmonellosis outbreaks due to inadequate or no cooking of the products before consumption. So they evaluated the effect of three antimicrobials against Salmonella during manufacture of a surface-browned uncooked frozen breaded chicken meat product. The products were breaded and surface browned by baking in an oven (208 C° for 15 minute) or deep frying in vegetable oil (190 C° for 15 second), packaged in polyethylene bags, and stored at -20 C° for 7 days. Previously, the results of Moschonas et al. (2012) were in agreement with the results in the current study as in the current study Salmonella

detected in all uncooked frozen breaded product (pane and drumsticks).

Also, according to Joekel et al. (1992) frozen chicken cuts were (12.9%), this percentage is lower than in of the current study (26.6%). Moreover, Goncalves et al. (1998) found that the percentages of the thigh and breast were 26.7% (4/15) in Brazil. Uyttendaele, De troy and Debevere (1999) detected Salmonella with percentages of (38.2%) in poultry samples. Also, Dufrenne et al. (2001) detected Salmonella from frozen chicken products (68%) which was higher than those of the current study (24.6%). While, Cetinkaya et al. (2008) found that by examination of the thigh and breast only one thigh sample (0.6%) was found to be contaminated with Salmonella. The isolated Salmonella strain was serotyped as (*S. infantis*), results disagreed with results of the current study as all product had positive Salmonella and thigh with percentage of 9.6%, and isolated Salmonella strains were serotyped as *S. enteritidis*, *S. typhimurium* and *S. anatum.* These results disagreed with the results of the current study.

Hassanein et al. (2011) collected 25 frozen chicken fillet samples as in the current study and Salmonella was detected in 13 samples (52%), these results were higher than those of fillet in the current study as in the current study Salmonella detected in 2 samples (8%). Abdellah et al. (2008) detected salmonella in breast with 6.25%. Jianghui (2014) detected Salmonella in the chilled stored poultry carcasses (55.1%) and the results were significantly higher in prevalence than frozen stored poultry carcasses (33.5%), while unpackaged (45.1%) was more likely to be contaminated with Salmonella than packaged (37.4%). Previously, unpackaged or chilled carcasses were in high risky which indicated a strong potential of the cross-contamination occurred at and/or before the retail level and the results were higher than frozen and package poultry carcasses in the current study (25.6%). And also, Nogueira et al. (2005) detected Salmonella chicken carcasses (69.7%) chilled and (30.3%) frozen. Dufrenne et al. (2001) isolated Salmonella from fresh chicken products (89%) and frozen products (68%). Vural et al. (2006) detected Salmonella in poultry carcasses 48% (12/25).

Javadi and Safarmashaei (2011) failed to isolate Salmonellae spp. from marketed broiler meat. Also, Lidija Kozačinski et al. (2006) detected Salmonella by percentages of 15.39% of chicken breast fillets without skin and in 9.52% of chicken breasts with skin.

Samaha et al. (2012) isolated Salmonella from pane (12%), this result was lower than the result in of current study (32%). Mahmoud and Hamouda (2006) isolated Salmonella in thigh and breast samples (2% each). Dominguez et al. (2002) isolated Salmonella in retail chicken meat samples (71) (35.83%) of the analyzed samples. The predominant serovars were *S. enteritidis* (47.88%), *S. hadar* (25.35%) and serotype 4, 12:b:-(II) (19.71%). other serovars such as *S. mbandaka*, *S. derby*, *S. virchow* and *S. paratyphi B* were isolated in much lower levels. Also, Salmonella organisms were isolated from (11.11%) chicken thigh by Nawar (2007), while, Ruban and Fairoze (2011) isolated 71.43 % for chicken thigh.

At the end, the obtained results in the current study allow to conclude that all samples (frozen raw packaged chicken cuts and frozen uncooked breaded packaged products) were contaminated with Salmonella. Concerning Salmonella spp. It is evident that *S. enteritidis*, *S. typhimurium*, *S. kentucky*, *S. anatum* and *S. haifa* were isolated from the examined chicken cut-up and products with difference percentages. Multiplex PCR was used in the current study to detect the virulence factors of S. *enteritidis* and *S. typhimurium* using four primers (Invasion A (InvA), Enterotoxin (*Stn), hyper-Invasive locus (*HilA) and Fimbrial (FimH) *genes*).

In order to minimize or prevent contamination of chicken meat (cuts-up) and chicken products by Salmonella and improve the sanitary status of chicken cut-

up processing and consequently the quality of chicken products, some recommendations should be carried out such as Application of the Good Hygienic Practices, GHPs, Good Manufacturing Practices, GMPs, and Hazard Analysis and Critical Control Point (HACCP) System in the Poultry processing operation.

5. REFERENCES

Abdellah, C., Fouzia, F.R., Abdelkadar, C., Rachida, B.S., Mouloud, Z.2008. Occurrence of Salmonella in chicken carcasses and giblets in Meknes-Morocco. Pak. J. Nutr. 7 (2):231-233.

Baumler, A.J., Tsolis, R.M., Heffron, F. 2000.Virulence mechanisms of Salmonella and their genetic basis. In: Salmonella in domestic animals. eds Wray, C., Wray, A. Wallingford, Oxford Shire, UK, CAB International, pp. 57–69.

Bean,N.H., Griffin,P.M.1990.Foodborne disease outbreaks in the United States, 1973-1987: Pathogens, Vehicles and trends. J. Food Prot. 53:804-817.

Cetinkaya, F., Cibik, R., Soyutemiz, G.E., Ozakin, C., Kayali, R., Levent, B. 2008. Shigella and Salmonella contamination in various foodstuffs in Turkey. Food Control. 19(11):1059-1063.

Clouthier, S.C., Muller, K.H., Doran, J.L., Collinson, S.K., Kay, W.W 1993.Characterization of three fimbrial genes, sef ABC of Salmonella enteritidis. J. Bacteriol. 175: 2523–2533.

Commission of the European Union, 2012. The Rapid Alert System for food and feed (RASFF): annual report 2011. http://ec.europa.eu/food/safety/rasff/docs/rasff_annual_report_2011_en.pdf.

Dominguez, C., Gomez, I., Zumalaca rregu, J. 2002. Prevalence of Salmonella and campylobacter in retail chicken meat in Spain. Inter. J. Food Microbiol. 72:165-168.

Dufrenne, J., Ritmeester, W., Delfgou-van Asch, E., Leusden, F. van, Jonge, R. de 2001. Quantification of the contamination of chicken and chicken products in the Netherlands with Salmonella and Campylobacter. J. of Food Prot. 64(4):538-541.

Duncan, M.J., Mann, E.L., Cohen, M.S., Ofek, I., Sharon, N., Abraham, S.N. 2005. The distinct binding specificities exhibited by entero bacterial Type 1 - Fimbriae are determined by their fimbrial shafts. J. Biol. Chem. 280:37707–37716.

GalanJEGinocchio, C., Costeas, P. 1992. Molecular and functional characterization of the Salmonella invasion gene invA: homology of invA to members of a new protein family. J. Bacteriol. 174: 4338-4349.

Goncalves, P.M.R., Franco, R.M., Zamborlini, L.C. 1998. Enumerac, ao de enterococos e coliforms fecais, pesquisa Salmonella e indicac, ao presuntiva de proteus, em cortes e miu dos de frango congelados. Hig. Aliment.12:42-47.

Hassanein, R., Ali, S.F.H., El-Malek, A.M.A., Moemen, A.M., Elsayh, K.I. 2011. Detection and identification of Salmonella species in minced beef and chicken meats by using Multiplex PCR in Assiut city. Veterinary World 4(1):5-11.

Hosam, A.H. Belal 1997. Microbiological studies of poultry cut-up products M. V. Sc. Fac. Vet. Med., Zagzig Univ.

ISO 6579:2002+A1:2007. Microbiology of food and animal feeding stuffs-Horizontal methods for detection of Salmonella species.

Javadi, A., Safarmashaei, S. 2011. Microbial profile of marketed broiler meat. Middle-East J. Sci. Res. 9(5):652-656.

Jianghui Zhu, Yeru Wang, Xiaoyu Song, Shenghui Cui, Haibin Xu, Baowei Yang, Jinlin Huang, Guihua Liu, Qian Chen, Gang Zhou, Qiuxia Chen, Fengqin Li, 2014. Prevalence and quantification of Salmonella contamination in raw chicken carcasses at the retail in China. Food Control 44:198-202

Joekel, J., Eisgruber, H., Klare, H.1992. Poultry meat in meat retail facilities. Assessment of Salmonella hazard. Fleischwirtschaft, 72(8):1135.

Kauffmann, F. 1974.Kauffmann White Scheme, WHO-BD/72, L, rev., 1. Acta. Path. Microbial. Scand. 61:385.

Lidija Kozačinski, Mirza Hadžiosmanović, Nevijo Zdolec, 2006. Microbiological

quality of poultry meat on the Croatian market. Vet. Arhiv 76:305-313.

Makino, S., KuraZono, H., Chongsanguam, M, Hyashi, H., Cheun, H., Suzuki, S., Shirahata, T. 1999. Establishment of the PCR system specific to Salmonella species and its application for the inspection of food and fecal samples. J. Vet. Med. Sci. 61: 1245–1247.

Mahmoud, Y.E., Hamouda, S.N. 2006. Quality evaluation of poultry meat carcass in El-Gharbia governorate markets. Assiut Vet. Med. J. 52(110):31-43.

Mohamed Karmi 2013. Detection of Virulence Gene (inva) in Salmonella Isolated from Meat and Poultry Products. Inter. J. of Genetics. 3(2):07-12.

Moschonas, G., Geornaras, I., Stopforth, J.D., Wach, D., Woerner, D.R., Belk, K.E., Smith, G.C., Sofos, J.N. 2012 Antimicrobials for reduction of Salmonella contamination in uncooked, surface-browned breaded chicken products. J. Food Prot. 75(6):1023-1028.

Nawar, A.Z. 2007. Correlation between Salmonella and sanitation level in poultry processing plants. M. V. Sc. Thesis (Meat Hygiene) Fac. Vet. Med. Benha Univ.

Ruban, S.W., Fairoze, N. 2011. Effect of processing condition on microbiological quality of market poultry meats in bengalore, Ind. J. Ani. Vet. Adv. 10(2):188-191.

Nogueira, N.A.P., Verde, J.C.L., Bas-tos, G.M., Brito, E.C.de O., Oliveira, M.T. de, Soares, M.I.M., Aguiar, A.C.L. 2005. Bacteria of the genus Salmonella in carcasses of chickens commercialized in Fortaleza, CE. Hygiene Alimentar; 19(137):87-89.

Samaha, I.A., Ibrahim, H.A.A., Hamat, M.O. 2012. Isolation of Enteropathogens from R Poultry Meat in Alexandria Alex. J. Vet. Sci 37(1):17-22.

Soomro, A.H., Khaskheli, M., Bhutto, M.B., Shah, G., Memon, A., Dewani, P. 2010. Prevalence and antimicrobial resistance of Salmonella serovars isolated from poultry meat in Hyderabad, Pakistan. Turkish. J. of Veterinary & Animal Sciences 34(5):455-460.

Tibaijuka, B., Molla, B., Hildebrandt, G., Kleer, J. 2003. Occurrence of Salmonellae in retail raw chicken products in Ethiopia. Berliner und Münchener tierärztliche Wochenschrift, 116(12):55-58.

Vural, A., Erkan, M.E and Yesilme, S. 2006. Microbiological quality of retail chicken carcasses and their products in Turkey. Medycyna Wet. 62(12):1371-1374.

Uyttendaele, M., De troy, P., Debevere, J.1999 Incidence of Salmonella, Campylobacter jejuni, Campylobacter coli and Listeria monocytogenes in poultry carcasses and different types of poultry products for sale on Belgian retail market. J. Food Prot. 62(7): 735−740.

Zadernowska, A., Chajecka, W. 2012. Detection of Salmonella spp. Presence in food, Salmonella. In: Salmonella- A Dangerous Foodborne Pathogen, Barakat, S. M. Mahmoud (ED.), ISBN: 978-953-307-782-6, In Tech. http://www.intechopen.com/books/Salmonella-a-dangerous-foodborne-pathogen/detection -of-Salmonella spp. presence-in food. (Accessed at. 2716/2015)