Design for Scientific RENAISSANCE

PURE

Occurrence of *Salmonella* in Fresh and Frozen Meat in the Gaza Strip

Abdelraouf A. Elmanama[1,a], Kamal A. E. El kahlout[2,b] and Shorok R. Elnakhalaa[3,c]

[1]Islamic University-Gaza, Medical laboratory Sciences Department, Gaza, Palestine.
[2]Islamic University-Gaza, Department of biology and Biotechnology, Gaza, Palestine.
[3]Islamic University-Gaza, Medical Laboratory Sciences Department, Gaza, Palestine.

[a]elmanama_144@yahoo.com

[b]kelkahlout@ iugaza.edu.ps

[c]shorok_biotechnologist@hotmail.com

Article Info

Received: 1st October 2013
Accepted: 25th November 2013
Published online: 1st December 2013

ISSN (Online): 2232-1179
ISSN (Print): 2314-8101

ABSTRACT

Salmonellosis is one of the most common and widely distributed food-borne diseases. It constitutes a major public health burden and represents a significant cost in many countries. This work has been conducted as a cross sectional design study to identify *Salmonella* occurrence in meat sold locally in Gaza strip, Palestine. For this purpose, a total of 150 fresh and frozen meat samples were collected. Samples were collected equally from the five governorates of Gaza strip (30 samples from each governorate). Samples were tested at the Public Health Laboratory of Ministry of Health (MOH) in Gaza using standard laboratory methods. The study showed that 139 (93.7%) of samples were negative for *Salmonella*, whereas 11 (7.3%) of samples were positive for *Salmonella*. The study showed that 13.3% of fresh chicken, 10% of fresh minced meat, 6.7% of frozen minced meat, 3.3% of fresh meat and 3.3% of frozen meat were contaminated with *Salmonella*. In addition, it was found that samples collected from Khan-Youns had the highest *Salmonella* contamination (13.3%) and

fresh chicken was of the most frequently contaminated with *Salmonella*. The study also showed that there was a statistically significant correlation between sample type and the presence of *S. aureus* (p=0.002) and Coliform (p=0.029), but there was no statistically significant correlation between sample type and Aerobic Plate Count (APC) (p=0.117) as well as *E. coli* (p=0.246). The study showed that there was no statistically significant correlation between Governorate and *Salmonella* (p=0.365), APC (p=0.626) and *E. coli* (p=0.167), but there was statistical significant between Governorate and *S. aureus* (p=0.014) and Coliform (p=0.011). The results also showed that there was statistically significance correlation between *Salmonella* and both APC and *S. aureus*, while, there was no statistically significant correlation between *Salmonella* and both Coliform and *E. coli*.In conclusion, the study demonstrated the presence of *Salmonella* in both fresh and frozen meat samples collected from the local markets in Gaza strip. Therefore, it is recommended to establish a permanent program for surveillance of *Salmonella* and all other food-borne pathogens.

***Keywords*: *Salmonella*, Detection, Fresh chicken, fresh meat, frozen meat, Gaza strip.**

1. Introduction

The genus *Salmonella* is a gram-negative rod-shaped bacteria which belongs to family *Entrobacteriacea*. It is a facultative intracellular pathogen that can cause diseases ranging from gastroenteritis to typhoid fever. Infections due to *Salmonella* serotypes continue to be a major health problem. Poultry and poultry products have been implicated as a major source of *Salmonella* infections in human as they still occur at high frequencies in industrialized nations and developing countries as well (Salehi *et al.*, 2005).

The typhoid caused by *Salmonella enterica* serotype Typhi remains an important public health problem in developing countries. In the year 2000, it was estimated that over 2.16 million episodes of typhoid occurred worldwide, resulting in 216,000 deaths and that more than 90% of this morbidity and mortality occurred in Asia. Furthermore, Salmonellosis causes substantial medical and economic burdens world-wide (Jyoti *et al.*, 2010). *Salmonella* infects over 160,000 individuals in the EU annually, with an incidence rate of 35 cases per 100,000. The annual cost of food-born Salmonellosis is believed to reach up to 2.8 billion € per year. Reports from World Health Organization surveillance program for control of food borne infections and intoxications in Europe revealed that the majority of outbreaks, were caused by *Salmonella* serotypes (McGuinness *et al.*, 2009).

Food-borne diseases are a widespread and growing public health problem affecting both developed and developing countries, microbiologically contaminated food and water being the major causes of diarrheal diseases. Although their global incidence is difficult to estimate, researchers generally agree in estimating that the percentage of the population suffering from food-borne diseases each year could be up to 30% in industrialized countries and could be even worse in developing countries (Germini *et al.*, 2009). According to the Centers for Disease Control and Prevention (CDC), *Salmonella* alone affects about 1.4 million people each year in the United States with about 16,000 hospitalizations and more than 500 deaths annually. In

1996, the USDA, Economic Research Service estimated that the total costs for medical care and lost productivity, resulting from food-borne *Salmonella* infections of humans was between 3.5-6 billion dollars annually. Control measures such as biosecurity practices, cleaning and disinfecting of facilities, rodent control programs, vaccination, and testing all can significantly increase production cost (Myint *et al.,* 2004).

The aim of this study is to evaluate occurrence of *Salmonella* species in fresh and frozen meat sold in the local markets of Gaza strip, Palestine in an attempt to generate first original data that might assist local authorities.

2. Materials and methods

2.1 Sample collection

The study was carried out through March to June, 2013 period. It has covered the five governorates of Gaza Strip area (North Gaza, Gaza City, Mid Zone, Khan Younis and Rafah). During study period, a total of 150 meat samples were collected from the five governorates of Gaza strip. The 150 samples were divided into five groups. Each group consisted from 30 samples representing one governorate. The thirty samples from each governorate included 6 samples of minced frozen meat, 6 samples of minced fresh meat, 6 samples of frozen meat, 6 sample of fresh meat, and 6 sample of fresh chicken. For each sample, 150 grams of meat type were collected in sterile bags.

2.3 Bacteriological analysis

2.3.1 Sample processing

Twenty five grams of each sample were homogenized at high speed in a stomacher with 225 ml peptone water (0.1 %) for 2 minutes. Ten fold serial dilutions were prepared under aseptic conditions from each sample using 0.1 % peptone water as diluent. The diluted samples were used within 10 minutes to perform the required analysis (FDA, 1998).

2.3.2 Aerobic plate count (APC)

Decimal dilutions (10^{-2}, 10^{-3} and 10^{-4}) of samples were used. One ml of each dilution was transferred aseptically into separate, Petri dishes in duplicate, 12-15 ml of melted (and cooled to 45 °C) count agar was added to each plate. The plates were swirled gently and after the agar medium solidified, the plates were incubated for 48±2 h at 35 °C (FAO/WHO Expert Committee on Food Additives, 2006).

2.2.3 Enumeration of Coliform

Decimal dilutions (10^{-2}, 10^{-3}, and 10^{-4}) of minced meat samples were used. One ml of each dilution was transferred aseptically into separate Petri dishes in duplicate. About 12-15 ml of melted (cooled to 45°C) Violet Red Bile Agar (VRBA) were added to each plate. The plates were swirled gently, left to solidify and then they were incubated for 18 to 24 h at 37°C. To prevent surface growth and spreading of colonies the plates were overlaid with 5 ml VRBA, and let to solidify (Feng *et al.,* 2002). Suspected colonies representing different types in accordance with their relative numbers were selected, and transferred to tubes of 2% Brilliant

green lactose bile (BGLB) broth (Wilson, 2001). The tubes were incubated at 35°C and were examined after 24 and 48 hours for gas production. Tubes producing gas were confirmed as coliform organisms. The number of coliforms per gram sample was calculated (Feng *et al.*, 2002).

2.2.4 Enumeration of *Staphylococcus aureus*:

Decimal dilutions (10^{-2}, 10^{-3}, and 10^{-4}) of samples were used. One ml of each dilution was transferred aseptically onto the surface of Baird Parker agar and was spread, using sterile bent glass spreading rod. Plates were incubated for 45-48 h at 35 ºC (FDA, 1998).

2.2.5 Isolation of *Salmonella*:

Pre-enrichment : Sample weight of 25g was suspended in 225 ml sterile lactose broth and blended for 2 min. Homogenized mixture was aseptically transferred to sterile wide-mouth, screw-cap jar (500 ml), and let to stand for 60 min at room temperature. Sample mixtures were incubated for 24 ± 2 h at 35 ºC (Andrews et al., 2011).

Enrichment: One ml pre-enrichment mixture was transferred to 10 ml selenite cystine (SC) broth and another 1 ml to 10 ml Tetrathionate broth (TT). SC and TT broths were incubated for 24 ± 2 h at 35 ºC.

Streaks from enrichment broth were carried out on Bismuth Sulfite (BS) agar, Xylose Lysine Desoxycholate (XLD) agar and *Salmonella-Shigella* (S.S) agar. Plates were incubated for 24 ± 2 h at 35 ºC. Plates were examined for presence of colonies resembling *Salmonella*. The streaks on xylose lysine desoxycholate (XLD) agar show pink colonies with or without black centers. The streaks on bismuth sulfite (BS) agar exhibited Brown, gray, or black colonies and sometimes they have a metallic sheen. The streaks on *Salmonella-Shigella* (S.S) agar resulted in black centered colonies.

2.2.5.1 Biochemical identification of isolates

The API-20E test kit for the identification of enteric bacteria (bio Merieux, Inc., Hazelwood, MO), Gram stain, catalase enzyme, coagualse and other common biochemical tests were used to confirm the identity of *S. aureus.*

2.2.5.2 Serological test

Polyvalent O Antisera and Polyvalent H Antisera for *Salmonella* were used. On a clean glass slide a drop of antiserum was deposited. Using a platinum loop a small amount of bacteria Agglutination is observed in the corresponding antiserum after a maximum of one minute.

3. Results

3.1 *Salmonella* isolation and identification

One hundred ninety three samples were negative for *Salmonella* and 11 (7.3%) samples were positive in Gaza strip (table 1).

Table 1 Percentage of *Salmonella* in meat samples

Salmonella	**Frequency**	**Percent**
Negative	139	93.7
Positive	11	7.3
Total	150	100.0

Regarding to *Salmonella*, the results showed almost a uniform geographical distribution (table 2). It was found that 3 (10%) in North Gaza, 2 (6.7%) in Gaza City, 2 (6.7%) in Middle Zone and 4 (13.3%) in Khan-Youns with no statistical significant differences (p value =0.365).

Table 2 Incidence rate of *Salmonella* in each Governorate

Governorate	***Salmonella***		**Total**
	Negative	**Positive**	
North Gaza	27 90.0%	3 10.0%	30 100.0%
Gaza City	28 93.3%	2 6.7%	30 100.0%
Middle Zone	28 93.3%	2 6.7%	30 100.0%
Khan-Youns	26 86.7%	4 13.3%	30 100.0%
Rafah	30 100.0%	0 .0%	30 100.0%
Total	139 92.66%	11 7.3%	150 100.0%

3.2 Correlation between sample type and prevalence of *Salmonella*

Table 3 shows the relation between sample type and incidence of *Salmonella*. It was found that eleven samples did not pass the standard specification and were positive for *Salmonella*, 4 (13.3%) in fresh chicken, 1 (3.3%) in fresh meat, 1 (3.3%) in frozen meat, 3 (10%) in fresh minced meat and 2 (6.7%) in frozen minced meat.

Table 3 Correlation between sample type and incidence of *Salmonella* (N=150)

Sample	***Salmonella***	
	Negative	**Positive**
Fresh chicken	26 86.7%	4 13.3%
Fresh meat	29 96.7%	1 3.3%
Frozen meat	29 96.7%	1 3.3%
Fresh minced meat	27 90.0%	3 10.0%
Frozen minced meat	28 93.3%	2 6.7%
Total	**139** **92.7%**	**11** **7.3%**

Results has revealed a significant correlation between sample type and *S. aureus* , and coliform while there was no significant differences between sample type and *E. coli* and APC (table 4).

Table 4 Correlation between sample type and microorganisms

Microorganisms	**Sample**	**P –value**
APC	Fresh chicken	0.117
	Fresh meat	
	Frozen meat	
	Fresh minced Meat	
	Frozen minced meat	
S. aureus	Fresh chicken	0.002
	Fresh meat	
	Frozen meat	
	Fresh minced meat	
	Frozen minced meat	
	Total	
Coliform	Fresh Chicken	0.029
	Fresh meat	
	Frozen meat	
	Fresh minced Meat	
	Frozen minced meat	
E. coli	Fresh Chicken	0.246
	Fresh meat	
	Frozen meat	
	Fresh minced Meat	
	Frozen minced meat	

Table 5 shows that there was a significant difference between Governorate and *S. aureus* and Coliform but there was no significant difference between Governorate and *Salmonella*, APC, *E. coli*.

Table 5 Correlation between indicator bacteria in each Governorate

Microorganisms	Governorate	P–value
APC	North	0.626
	Gaza	
	Middle	
	Khan-Youns	
	Rafah	
	Total	
S.aureus	North	0.014
	Gaza	
	Middle	
	Khan-Youns	
	Rafah	
	Total	
Coliform	North	0.011
	Gaza	
	Middle	
	Khan-Youns	
	Rafah	
E. coli	North	0.167
	Gaza	
	Middle	
	Khan-Youns	
	Rafah	

In Table 6 there was a significant correlation between APC level and *Salmonella* (p value = 0.034).

Table 6 Correlation between APC level and *Salmonella* (N=150)

Salmonella	APC **interval**		
	0-10000	**10001-100000**	**over 100000**
Negative	67 48.2%	60 43.2%	12 8.6%
Positive	1 9.1%	9 81.8%	1 9.1%
Total	68 45.3%	69 46.0%	13 8.7%

(p value = 0.034)

In Table 7 there was a significant correlation between *Salmonella* and *S. aureus* level.

Table 7 Correlation between *S. aureus* level and *Salmonella* (N=150)

Salmonella	***S. aureus* levels**		
	0-100	**101-200**	**Over 200**
Negative	127 91.4%	1 .7%	11 7.9%
Positive	7 63.6%	0 .0%	4 36.4%
Total	134 89.3%	1 .7%	15 10.0%

(p value = 0.010)

4. Discussion

The bacteriological investigation of 150 meat samples, revealed that only 11 (7.3%) were positive. Results of the study showed that Khan-Youns area had the highest contamination rate of *Salmonella* which is 13.3%. This finding may be explained by the presence of large agricultural representing about 91.9% out of the governorate total area according to Palestinian Central Bureau of Statistics (PCBS, 2010). In these areas, chemical and organic fertilizers are widely used. This may be a possible source of contamination since organic fertilizers are rich source of bacteria and constitute a suitable growth media for microorganism. In addition to that, Khan-Youns governorate lack of sewage networks and sewage can leaks to agricultural areas and even to ground water.

The present work showed a statistical correlation between the occurrence of *Salmonella* and contamination with *S. aureus*. This finding may be related to food miss-handling by workers as reported by the Michigan Department of Agriculture which concluded that the presence of *S. aureus* is considered as an indicator of handling abuse (Michigan Department of Agriculture-MDA. 2005).

The present work indicated a relationship between APC and *Salmonella* presence and this relation was statistically significant. This finding is in disagreement with Castillo–Ayala, *et al.*, (1993) where they detected no relationship between APC and *Salmonella* isolated from fresh chicken. The study showed there was a statistical significant relationship between the presence of *S. aureus*, Coliform and sample type. This result may be due to presence of *S. aureus* as normal flora on the surface of animals.

Sobukola *et al.,* (2009); Okonko *et al.,* (2008 a,b,c,d, 2009 a,b) mentioned that they were not able to isolate *S. aureus* in their studies, and the occurrence of *E. coli* in (11.1%) of fresh meat samples is an indication of fecal contamination. This might be due to possible contamination of fresh meats or meat products during sales or unhygienic handling of animals and meat during slaughtering, butchering and processing or due to contamination from the skin, mouth, or nose of the handlers which can be introduced directly into foods by workers having lesions caused by *S. aureus* on hands and arms, or by coughing and sneezing.

Okonko *et al.,* (2009 a,b) reported that *Salmonella* with (11.1%) incidence was found in meats and this is of public health significance and concerns. The isolation of *Salmonella* in their study was considered of practical impact. They suggested that this organism might have contaminated meats as a result of handling by meat sellers.

This is also in accordance to the assertion of Okonko *et al.,* (2008d, 2009a,b) that improper handling and improper hygiene might lead to the contamination of ready-to-eat foods and this might eventually affects the health of the consumers. This was illustrated by the presence of the indicator organisms.

In conclusion, this study showed that 11 (7.3%) of samples were positive for *Salmonella*. The study showed that fresh chicken, fresh minced meat, frozen minced meat, fresh meat and frozen meat were contaminated with *Salmonella*, 13.3%, 10%, 6.7%, 3.3% and 3.3% respectively. It is recommended to establish a permanent program for surveillance of *Salmonella* and all other food-borne pathogens. And to do further studies to identify levels of *Salmonella* serotypes occurrence in Palestine.

.

Reference

Andrews, W., Jacobson, A., and Hammack, T. 2011. Bacteriological Analytical Manual *Sallmonella*.

Castillo-Ayala, A., Salas-Ubiarco, M.G., Marquez-Padilla, M. L and Osorio-Hernadez, M. D. 1993. Incidence of *Campylobacterspp.* and *Salmonellas pp.* in raw and roasted chicken in Guadalajara, Mexico. Rev Latinoam Microbiologia, 35(4) 371-375.

FDA BAM, 1998. FDA Bacteriological Analytical Manual. Chapter 7, *Campylobacte*r, Hunt, J.M., Abeyta, C. & Tran, T. 8th edition (revision A), 23 pages.

Feng, P., Weagent, S. D and Grant, M.A., 2002. Bacteriological Analytical Manual. available Online: www.lib.ncsu.edu/pubweb/www/ETDdb/web_root/collection/available/etd-04102005-213953/ unrestricted/ etd. pdf.

Germini, A., Masola, A., Carnevali, P., Marchelli, R., 2009."Simultaneous detection of *Escherichia coli* O175:H7, *Salmonella spp*, and *Listeria monocytogenes*by multiplex PCR". Food Control 20 733–738.

Indiana State Department of Health, 2009. Available online: http://www.state.in.us/isdh/files/-diseases.pdf.

Joint FAO/WHO Expert Committee on Food Additives. Combined Compendium of Food Additive Specifications. Volume 4. Analytical methods, test procedures and laboratory solutions used by and referenced in the food additive specifications. 2006. Food and Agriculture Organization of the United Nations, Rome.

Jyoti, A., Ram, S., Vajpayee, P., Singh, G., Dwivedi, P.D, Jain, S.K, Shanker, R. 2010. "Contamination of surface and potable water in South Asia by *Salmonella*: Culture-independent quantification with molecular beacon real-time PCR". Science of the Total Environment, 408:1256–1263.

Mc Guinness, S. H., McCabe, E., O'Regan, E., Dolan, A., Duffy, G., Burgess, C., Fanning, S., Barry, T., O'Grady, J., 2009. "Development and validation of a rapid real-time PCR based method for the specific detection of *Salmonella* on fresh meat".Meat Science, 83: 555–562.

Michigan Department of Agriculture-MDA. 2005. Training Program for the Professional Food Service Sanitarian Module 6: Foodborne Microbiological Control. Available online: www.michigan.gov /documents.

Myint, M. S, 2004. "Epidemology of *Salmonella* Contamination of Poultry Meat Products": Knowledge Gaps In TheFarmto Store Products.

Okonko, I.O., Adejoye, O.D., Ogunnusi, T.A., Fajobi, E.A., Shittu, O.B., 2008a. Microbiological and physicochemical analysis of different water samples used for domestic purposes in Abeokuta and Ojota, Lagos State, Nigeria. African J. Biotechnology,7(3): 617-621.

Okonko, I. O., Ogunjobi, A. A., Fajobi, E. A., Onoja, B. A., Babalola, E. T., Adedeji, A. O., 2008b. Comparative studies and microbial risk assessment of different Ready-to-Eat (RTE) frozen sea-foods processed in Ijora-olopa, Lagos State, Nigeria. African J. Biotech, 7(16): 2898-2901.

Okonko, I. O., Ogunjobi, A. A., Adejoye, O. D., Ogunnusi, T.A, Olasogba, M.C., 2008c. Comparative studies and Microbial risk assessment of different water samples used for

processing frozen sea-foods in Ijoraolopa, Lagos State, Nigeria. African J. Biotechnolo.,; 7(16): 2902-2907.

Okonko, I. O., Ogunnusi, T. A., Ogunjobi, A. A., Adedeji, A. O., Adejoye O.D., Babalola, E.T., Ogun A.A., 2008d. Microbialstudies on frozen shrimps processed in Ibadan and Lagos, Nigeria. Scientific Research and Essay, 3(11):537-546.

Okonko, I. O., Ogun, A. A., Adejoye, O. D., Ogunjobi, A. A., Nkang, A. O., Adebayo-Tayo, B.C., 2009a. Hazards analysis critical control points (HACCP) and Microbiology qualities of Sea-foods as affected by Handler's Hygiene in Ibadan andLagos, Nigeria. African Journal of Food Science, 3(1):035-050.

Okonko, I.O., Donbraye, E., Babatunde, S.O.I., , 2009b. Microbiological Quality of Seafood processors and water used in two different sea processing plants in Nigeria EJEAFche, 8(8): 621-629.

Salehi, T.Z., Mahzounieh, M and Saeed zadeh, A., 2005. "Detection of InvA Gene in Isolated *Salmonella* from Broilers by PCR Method". International Journal of Poultry Science 4 (8): 557-559.

Sobukola, O. P., Awonorin, O. S., Idowu, A. M., Bamiro, O. F., 2009. Microbial profile and critical control points during processing of 'robo' snack from melon seed (*Citrulluslunatus*thumb) in Abeokuta, Nigeria. African Journal of Biotechnology, 8(10):2385-2388.

Wilson, P, 2001. Determination of coliforms in foods using violet red bile agar. MFHPB-31.Health Products and Food Branch, Microbiology Evaluation Division Bureau of Microbial Hazards, Health Canada, Ottawa, Ontario, Canada. Available online: http://www.pcbs.gov.ps/