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Molecular Diagnosis of *Salmonellae* in Frozen Meat and Some Meat Products

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Abstract

A total of one hundred and twenty random samples of luncheon, fresh raw sausage, frozen packed minced meat and frozen meat (30 samples of each) were collected from different supermarkets at Kalyobia Governorate. The collected samples were transferred directly to the laboratory in an ice box under complete aseptic conditions. The samples were immediately examined bacteriologically for the detection of *Salmonellae.* The *Salmonellae* isolates were confirmed by PCR.

Salmonellae failed to be detected in luncheon beef samples but the percentage of *Salmonellae* in fresh sausage was 10% and the isolated serovars were *S. typhi* (3.3%), *S. typhimurium* (3.3%) and *S. enteritidis* (3.3%). In frozen packed minced meat, the percentage of *Salmonellae* was 6.7% and the isolated serovars were *S. typhi* (3.3%) and *S. typhimurium* (3.3%) and in frozen meat, the percentage of *Salmonellae* was 13.3% and the isolated serovars were *S. papuana* (6.7%), *S. paratyphi A* (3.3%) and *S. vircho* (3.3%). The virulence of the isolated strains was confirmed through molecular technique by polymerase chain reaction (PCR) of the isolated *Salmonellae* strains for detection of the virulence factor (*invA* gene).

Keywords: Luncheon; Sausage; Minced meat; Frozen meat; *Salmonellae;* Serovars; PCR

Introduction

Food borne illnesses are considered by the World Health Organization (WHO) as diseases either infectious or toxic made by causative agents in ingested food. The reports in 2005 recorded 1.8 million people died from diseases causing diarrhea and high proportion of which was attributed to contamination of food and drinking water [1]. *Salmonellae* food poisoning is one of the most common and widely distributed diseases in the world [2], estimated to cause 1.3 billion cases of gastroenteritis and 3 million deaths worldwide [3].

Recently, some food borne diseases are considered as emerging diseases. Various food borne pathogens have been identified for food borne illness. *Campylobacter, Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonellae* are found to be responsible for most of food origin outbreaks [4].

Meat products such as sausage, luncheon and minced meat are more popular because they are considered as quick easily prepared meat meals and solve the problem of fresh meat shortage which is not within the reach of many peoples.

Microorganisms may contaminate meat products during a long chain of processing from the time of preparation, handling, processing, distribution and storage as well as marketing. Such contamination may render the products of inferior quality or even unfit for human consumption and at times may constitute a public health hazard. The possibility of contamination of meat products with food poisoning bacteria especially *Salmonellae* organisms has been extensively reported [5].

Traditional and conventional methods for identification of microbial pathogens depend on specific bacteriological and biochemical identification [6], which are time consuming, laborious and less accurate [7] and slow because *Salmonellae* needs 5-7 days to confirm its presence in meat products [8]. Several Polymerase Chain Reaction (PCR) assays have been developed by targeting various *Salmonellae* genes, such as *invA* [9,10]. The *invA* gene, is widely used as a target in PCR assays for *Salmonella* detection [11-13].

Materials and Methods

Isolation and identification of *Salmonellae*

The techniques adopted were carried out according to ICMSF (1978) [14]. Samples were cultivated for the isolation of *Salmonellae* species on MacConkey agar. After 24 h of incubation at 37°C, suspected colonies with typical characteristics of *Salmonellae* were sub-cultured on XLD (Xylose Lysine Deoxycholate) agar for 24 h at

37°C and *Salmonella shigella* (S.S) agar plates then incubated at 37°C for 24 h.

Molecular identification of *Salmonellae* isolates by PCR

Extraction of genomic DNA according to QIAamp DNA mini kit instructions: Genomic DNA was extracted from cell suspensions of bacteria grown overnight on XLD broth at 37°C using QIAamp® DNA Mini kit from Qiagen according to Lee SH, et al. [15]. A single colony from *Salmonellae* was resuspended in 3 ml XLD liquid media and grown overnight at 37°C. The bacterial culture was precipitated by centrifugation in a microcentrifuge at 5000 xg for 10 min. The bacterial pellets were resuspended in 180 µl AL buffer (supplied in the QIAamp DNA Mini Kit) for complete lysis. The samples were immediately cooled on ice for 5 min, and the cell lysates were digested with 20 µl of proteinase K (final concentration 800 µg/ml) at 56°C for 3 h. 200 µl ethanol (96 to 100%) was added to each tube and vortexed for 15 s. The mixtures were loaded onto the QIAamp Mini spin column and centrifuged at 6000 xg for 1 min. The filtrate was discarded and the spin column was placed in a clean collection tube (2 ml). A 500 µl of AW1 wash buffer was added to the QIAamp spin column and centrifuged at 6000 xg for 1 min. The washing step was repeated twice using 500 µl of washing buffer AW2. To elute bacterial DNA, 100 µl elution buffer (AE) was added to the center of the column, and the column was incubated at 37°C for 5 min then centrifuged at 20,000 xg for 1 min. DNA purity and quantity was determined using spectrophotometer (GeneSys 10UV, Thermo Scientific, USA).

Oligonucleotide primers for *Salmonellae* spp. (*invA* gene): Primer sets for the pathotypes and virulence genes for the isolated *Salmonellae* spp. according to Oliveira, et al. [16] (Table 1).

DNA amplification: The samples were done in BioRad Thermal cycler (T100-England) with the following thermal profile: initial denaturation at 95°C for 5 min then denaturation at 95°C for 30 sec, annealing at 60°C for 30 sec, extension for 30 sec at 72°C for 35 cycles and after the last cycle, the mixture was incubated for final extension at 72°C for10 min.

Detection of PCR products according to Sambrook J, et al. [17]: Amplified products were detected in 1.5% agarose gel electrophoresis pre-stained with ethidium bromide, at 80 V for 1 hour. Specific amplicons were observed under ultraviolet transillumination, compared with the marker, the gel was transferred to UV cabinet, and the gel was photographed by a gel documentation system and the data was analyzed through computer software.

Results and Discussion

In this work, a total of one hundred and twenty random samples of meat products, (30 samples of luncheon beef, 30 samples of fresh sausage, 30 samples of frozen minced meat and 30 samples of frozen meat) were examined for *Salmonellae*. The virulence of the isolated serotypes was confirmed by detection of the *invA* gene which is responsible for the pathogenicity of *Salmonella* serotypes.

Incidence of *Salmonellae*

Table 1: Primers used for the detection of *Salmonellae* sp.

Primer	Sequence	Amplified product
invA	GTGAAATTATCGCCACGTTCGGGCAA	284 bp
	TCATCGCACCGTCAAAGGAACC	

Salmonellae were detected in 7.5 % of the examined meat product samples. The percentage of *Salmonellae* in luncheon meat, fresh sausage, frozen minced meat and frozen meat was 0%, 10% and 6.7% and 13.3% respectively (Table 2).

The results present in table 2 revealed that *Salmonellae* failed to be detected in the examined luncheon samples.

These results agree with that reported by Gouda HI, Saleh AA, Edris A, Nashed-Heba F, Moussa MM, et al, Fathi S, et al, Aiedia HA, Abd-El-Aziz A, S, et al., Ouf-Jehan M, Eleiwa-Nesreen ZH, Edris AM, et al, Abd El-Kader-Hanaa A, et al. and Shaltout FA, et al. [18-30]. The result disagrees with that reported by Mohamed (1988) [31] who recorded that *Salmonellae* could be detected out of 100 examined luncheon samples and Mohamed K [32] who recorded that *Salmonellae* could be detected out of 10 examined luncheon samples (10%).

The results presented in table 2 revealed that *Salmonellae* were present in 10% of the examined samples of fresh sausage. Similar results were recorded by Edris AM, et al. [28]. Nearly similar results were obtained by Ouf-Jehan M, Eleiwa-Nesreen ZH and Mohamed FA [26,27,33] with an incidence of (8%) for each of them, Cabedo L, et al. (11.1) and El-Kader HA, et al. (13.3) [34,29]. While higher results were reported by Banks JG, et al. [35] (55 %) and Shaltout FA, et al. (16%) [30]. Lower results obtained by Zaki EMS (5%) and Ahmed AAH, et al. (5 %) [36,37]. Some investigators failed to detect *Salmonellae* in meat products as Vazgecer B, et al. and Ismail AH [38,39].

It's clear from the results reported in table 2 that 6.7% of the examined samples of frozen packed minced meat were positive for *Salmonellae*. This result agrees with that of Edris AM, et al. and El-Kader HA, et al. [28,29].

Nearly similar results were obtained by Abd El-Aziz AS, et al. (5%), and Shaltout FA (6%) [25,40]. While lower results were recorded by Roberts TA, et al. and Abd El-Atty NS, et al. [41,42] as (2%) for each of them. On the other hands, higher results were reported by Molla B, et al. (32.7%), Ejeta G, et al. (14.4%), Mohamed K (40%), AL-Jobori KM, et al. (36%), and Shaltout FA, et al. (40%) [43,44,32,7,30].

Salmonellae were present in 13.3 % of the examined samples of frozen meat (Table 2). Nearly similar results were recorded by El-Kader HA, et al. [29] (10%), but higher results reported by AL-Jobori KM, et al. [7] (40%) and Mahmood NR, et al. [45] who isolated *Salmonellae* spp. at a rate of 24.76% and Elsayed MS, et al. (18%) [46].

From the results recorded in table 2 it's clear that frozen meat had the higher incidence of *Salmonellae* contamination followed by fresh sausage and frozen packed minced meat while *Salmonellae* could not be detected in luncheon.

The absence of *Salmonellae* in luncheon meat may be due to the addition of food additives such as spices and preservatives, which have

Table 2: Incidence of *Salmonellae* in the examined meat products and frozen meat samples.

Samples	No. of samples	Positive	%
Luncheon (beef)	30	0	0
Fresh sausage	30	3	10
Frozen packed minced meat	30	2	6.7
Frozen meat	30	4	13.3
Total	120	9	7.5

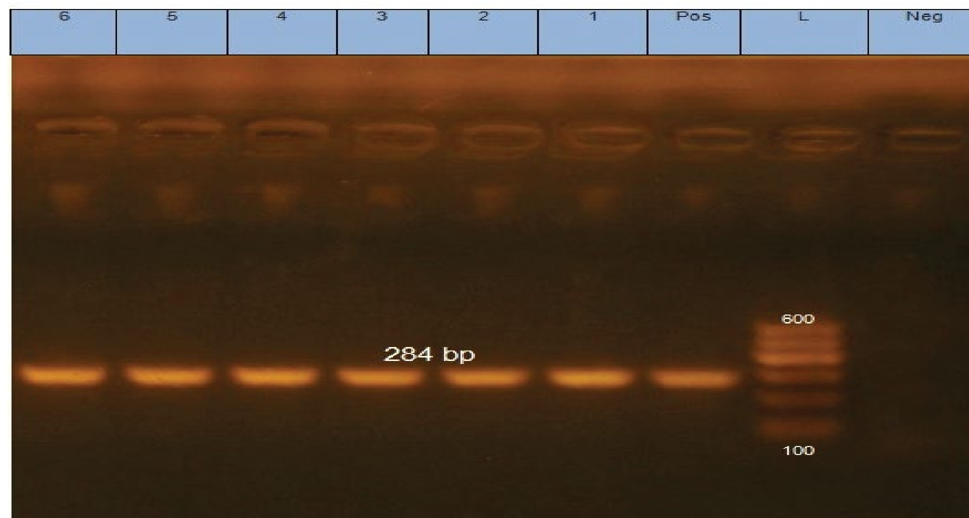

Figure 1: Agarose gel electrophoresis 2% showing PCR products of *invA* gene (284 bp) Specific for characterization of all *Salmonella* species. Lanes (1) to (6): Positive *Salmonella* species for *invA* gene.

Lane L: Molecular marker DNA ladder (100 bp) (cat. No. 239035) supplied from QIAGEN (USA)
Lane Pos: Control positive *Salmonella* strain for *invA* gene
Lane Neg: Control negative
Lane 1: *Salmonella typhi*
Lane 2: *Salmonella typhimurium*
Lane 3: *Salmonella enteritidis*
Lane 4: *Salmonella paratyphi A*
Lane 5: *Salmonella papuana*
Lane 6: *Salmonella vircho*

an antimicrobial activity and inhibit survival and multiplication of micro-organisms [47]. This also may be attributed to the exposure to high temperature during processing and cooking procedures.

High incidence of *Salmonellae* in fresh sausage may be due to faults in certain practices of slaughtering and handling processes such as the use of contaminated knives, tools, rags, saws, boards, etc., as well as unhygienic slaughtering, dressing, washing, transporting, handling and cutting in abattoirs and butcher shops that affect the incidence of *Salmonellae*. High incidence of *Salmonellae* in fresh sausage may be due to the fact that this product is made from raw meat in addition to natural casing which is often used in their manufacture and may be act as an important source for *Salmonella* [48].

The high incidence of *Salmonellae* in frozen packed minced meat may be due to cutting and contamination of meat besides the increase in its water and oxygen contents as well as contamination from grinders, air, packaging materials and hands of the workers. Temperature rise (2-4°C) during grinding could also increase the incidence of *Salmonella* organisms [49].

The incidence of *Salmonellae* in frozen meat may be originated through a different infected touch of food handlers and butchers, improper clothing, fecal hand contamination, may be through rodent and other insects or unhygienic practice in slaughter house area. There is a risk of chances for developing Salmonellosis is major due to survival of *Salmonella* at very low temperature too. They may come in different serotypes *S. enteridis, S. typhi, S. typhimurium*, etc. [50].

Serotyping of the isolated *Salmonellae*

From the result recorded in table 3, it is clear that 3 *Salmonella* serovars were identified from fresh sausage samples, 1 (3.3 %) strains as *S. typhi*, 1 (3.3 %) strains as *S. typhimurium* and 1 (3.3%) strain as *S. enteritidis.*

Similar results were obtained by Abd EL-Kader-Hanaa, et al. [29] where they could isolate *S. typhi* and *S. typhimurium* with a percentage of 3.3 % for each strain but higher results were recorded for *S. enteritidis* with a percentage of 6.7%.

Nearly similar results were obtained by Rao NM, et al. [51] where they could isolate *S. typhimurium* and *S. enteritidis* with a percentage of 2.5 % for each strain, Edris AM, et al. [28] where they could isolate *S. typhi*, *S. typhimurium* and *S. enteritidis* with a percentage of 4%, 4% and 2% respectively and Shaltout FA, et al. [30] where they could detect *S. enteritidis* (4%).

From the result recorded in table 4 it is clear that 2 *Salmonellae* serovars were identified in the examined frozen packed minced meat samples and identified as 1 (3.3%) strain as *S. typhimurium* and 1 (3.3%) strain as *S. typhi*.

This result agrees with that obtained by El-Kader HA, et al. [29] where they recorded that the incidence of *S. typhimurium* in the examined frozen packed minced meat samples was 3.3 %.

Nearly similar results were obtained by Edris AM, et al. [28] where they could isolate *S. typhi* (2%) and *S. typhimurium* (4%) and Shaltout FA, et al. [30] where they could detect *S. typhi* (4%), but higher results were recorded for *S. typhimurium* (8%).

From the result recorded in table 5 it's clear that 3 *Salmonella* serovars were identified in the examined frozen meat samples and identified as 2 (6.7%) strains as *S. papuana*, 1 (3.3%) strain as *S. paratyphi A* and 1 (3.3%) strain as *S. vircho*. Higher results were reported by Elsayed MS, et al. [46] where they stated that *S. paratyphi A* was detected in 8/18 (44.44%) positive samples.

Identification of *Salmonella* by PCR

The specificity of the oligonucleotide primers were carried out by testing of all *Salmonella* strains with PCR using the primer pairs targeting the *invA* gene (specific for all members of *Salmonella* species).

All the 6 *Salmonella* isolates detected by bacteriological examination were tested by PCR using the same primer pair after selective enrichment media on XLD agar and all *Salmonella* serovars were positive for amplification of 284 bp fragments of *invA* gene as shown in figure 1.

The present study supports the ability of this specific primer set to confirm the isolates as *Salmonella*. Isolates were subjected to *Salmonella* specific gene (*invA*) and were confirmed as *Salmonella* positive by the predicted product of 284 bp DNA fragments. The results obtained in the present study were in accordance with Nagappa K, et al., Abd El-Kader-Hanaa A, et al., and Moustafa NY, et al. [52,29,53] where they could amplify 284 bp of *invA* gene which is specific for demonstration and characterization of the isolated *Salmonella* species by using PCR.

The ability of *Salmonella* specific primers to detect *Salmonella* species rapidly and accurately in the present study is primarily due to the primer sequences that are selected from the gene *invA* of *S. typhimurium* as reported by Darwin KH, et al., Nucera DM, et al., and Craciunas C, et al. [54-56].

Table 3: Serotyping of *Salmonellae* isolated from the examined fresh sausage samples (n=30).

Salmonella serovars	No.	%*	%**
S. typhi	1	33.3	3.3
S. typhimurium	1	33.3	3.3
S. enteritidis	1	33.3	3.3

%*=percentage collected from only positive *Salmonella* samples
%**=percentage collected from total examined samples

Table 4: Serotyping of *Salmonellae* isolated from the examined frozen packed minced meat samples (no=30).

Salmonella serovars	No.	%*	%**
S. typhi	1	50	3.3
S. typhimurium	1	50	3.3

%*=percentage collected from only positive *Salmonella* samples
%**=percentage collected from total examined samples

Table 5: Serotyping of *Salmonellae* isolated from the examined frozen meat samples (n=30).

Salmonella serovars	No.	%*	%**
S. papuana	2	50	6.7
S. paratyphi A	1	25	3.3
S. vircho	1	25	3.3

%*=percentage collected from only positive *Salmonella* samples
%**=percentage collected from total examined samples

Culture techniques are universally recognized as the standard methods for the detection of bacterial pathogens, such as *Salmonella* in food stuffs [57]. These techniques generally take longer time [58] and are less sensitive compared to PCR based methods [55,56]. The use of *invA* gene specific PCR method in most diagnostic and research laboratories is possible and through the molecular basis of *Salmonella* identification techniques, this method is the simplest and less expensive.

Conclusion

In conclusion, PCR is a rapid and specific method for the detection of the virulent strains of *Salmonellae* in meat products and frozen meat samples. It gives the ability to detect *Salmonella* cells and identify the virulence of the isolated strains within a little time and PCR was demonstrated to be accurate methods for *S. enterica* identification.

References

1. World Health Organization (WHO) (2007) Food Safety and Food-Borne Illness-Fact Sheet N°237. Geneva, Switzerland.
2. World Health Organization (WHO) (2018) *Salmonella* (non-typhoidal). Geneva, Switzerland.
3. Bhunia AK (2008) Foodborne Microbial Pathogens: Mechanisms and Pathogenesis. Springer Science & Business Media, USA.
4. Chemburu S, Wilkins E, Abdel-Hamid I (2005) Detection of Pathogenic Bacteria in Food Samples Using Highly-Dispersed Carbon Particles. Biosens Bioelectron 21: 491-499.
5. Abd El-Aziz-Reham A (2004) Microbial evaluation of some meat products. MVSc Thesis (Meat hygiene) Fac Vet Med Moshtohor Zagazig University (Benha Branch), Egypt.
6. Aycicek H, Aydogan H, Kucukkaraaslan A, Baysallar M, Basustaoglu AC (2004) Assessment of the Bacterial Contamination on Hands of Hospital Food Handlers. Food Control 15: 253-259.
7. AL-Jobori KM, AL-Bakri AK, AL-Baity BH (2015) Detection of *Salmonella* spp. in Different Food Sources in Baghdad City: A Comparison between Conventional and Chromogenic Methods. Int J Adv Res Biol Sci 2: 171-184.
8. Helmy-Noha A (2003) Application of biotechniques for detection and discrimination of some pathogenic microorganisms in chicken meats. PhD Thesis (Meat Hygiene), Fac Vet Med, Cairo University, Egypt.
9. Rahn K, De Grandis SA, Clarke RC, McEwen SA, Galán JE, et al. (1992) Amplification of an *invA* Gene Sequence of *Salmonella typhimurium* by Polymerase Chain Reaction as a Specific Method of Detection of *Salmonella*. Mol Cell Probes 6: 271-279.
10. Moussa IM, Gassem MA, Al-Doss AA, Mahmoud WAS, Mawgood AAL (2010) Using Molecular Techniques for Rapid Detection of *Salmonella* Serovars in Frozen Chicken and Chicken Products Collected from Riyadh, Saudi Arabia. Afr J Biotechnol 9: 612-619.
11. Hara-Kudo Y, Yoshino M, Kojima T, Ikedo M (2005) Loop-Mediated Isothermal Amplification for the Rapid Detection of *Salmonella*. FEMS Microbiol Lett 253: 155-161.
12. Singer RS, Cooke CL, Maddox CW, Isaacson RE, Wallace RL (2006) Use of Pooled Samples for Detection of *Salmonella* in Feces by Polymerase Chain Reaction. J Vet Diagn Invest 18: 319-325.

13. Shanmugasamy M, Velayutham T, Rajeswar J (2011) *InvA* gene specific PCR for detection of *Salmonella* from broilers. Vet World 4: 562-564.
14. International Commission on Microbiological Specification for Foods (1978) Microorganisms in Foods 1: Their Significance and Methods of Enumeration: A Publication of the International Commission on Microbiological Specifications for Foods (ICMSF) of the International Association of Microbiological Societies. 2nd Edition, University of Toronto press 434.
15. Lee SH, Jung BY, Rayamahji N, Lee HS, Jeon WJ, et al. (2009) A Multiplex Real-Time PCR for Differential Detection and Quantification of *Salmonella* spp., *Salmonella enterica* Serovar *Typhimurium* and *Enteritidis* in Meats. J Vet Sci 10: 43-51.
16. Olivera SD, Rodenbusch CR, Ce MC, Rocha SLS, Canal CW (2003) Evaluation of Selective and Non Selective Enrichment PCR Procedures for *Salmonella* Detection. Lett Appl Microbiol 36: 217-221.
17. Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: A Laboratory Manual, 2nd Edition. Cold spring Harbor Laboratotry press, New York, USA.
18. Gouda HI (1991) Indicator Organisms in Some Meat Products. MVSc Thesis (Meat Hygiene), Fac Vet Med, Alexandria University, Egypt.
19. Saleh AA (1991) Hygienic and Economic Aspects of Some Microorganisms Affecting Production and Quality of Some Meat Products. MVSc Thesis (Meat hygiene), Fac Vet Med, Alexandria University, Egypt.
20. Edris A (1993) Isolation and Identification of *E. coli* and *Salmonella* from Ready to Eat Meat Products. Zag Vet Med J 21: 187-193.
21. Nashed-Heba F (1993) *Salmonella* and Enteropathogenic *E. coli* serotypes in meat products. MVSc Thesis (Meat Hygiene), Fac Vet Med, Moshtohor Zagazig University (Benha Branch), Egypt.
22. Moussa MM, Awad HA, Yassien MM, Gouda H (1993) Microbial Quality of Some Meat Products. Alex Vet Med J 41: 59-62.
23. Fathi S, El-khateib T, Moustaf S, Hassanein K (1994) *Salmonellae* and Enteropathogenic *E.coli* in Some Locally Manufactured Meat Products.
24. Aiedia HA (1995) Quality Investigation into Room Kept Traditional Meat Products in Egypt. PhD Thesis (Meat Hygiene), Fac Vet Med, Cairo University, Egypt.
25. Abd El-Aziz AS, El-Neklawy EIS, Hussien A, Niazi Z (1996) Food Poisoning Microorganisms in Some Locally Meat Products. Vet Med J Giza 44: 691-698.
26. Ouf-Jehan M (2001) Microorganisms of Sanitary Importance in Some Meat Products and their Additives. PhD Thesis (Meat Hygiene), Fac Vet Med, Cairo University, Egypt.
27. Eleiwa-Nesreen ZH (2003) Effect of Chemical Preservatives on Food Poisoning Bacteria in Some Locally Manufactured Meat Products. PhD Thesis (Meat hygiene), Fac Vet Med, Zagazig University, Egypt.
28. Edris AM, Shaltout FA, Salem GH, El-Toukhy EI (2011) Plasmid Profile Analysis of *Salmonellae* Isolated from Some Meat Products. Benha Vet Med J 172-178.
29. El-Kader HA, EL-Toukhy EI, Hanan AF, Masoud EA, EL-Berbawy SM (2015) Molecular Study on Virulence Gene of Some Isolates of *Salmonellae* Isolated from Chicken Meat and Some Meat Products. Anim Health Res J 3: 310-317.
30. Shaltout FA, Salem-Amani M, Khater-Dalia F, Lela-Radwa A (2016) Studies on Bacteriological Profile of Some Meat Products. Benha Vet Med J 31: 43-49.
31. Mohamed AS (1988) *Salmonella* in Locally Produced Meat Products. MVSc thesis (Meat Hygiene) Faculty of Veterinary Medicine, Cairo University, Egypt.
32. Mohamed K (2013) Prevalence of *Salmonella* in Meat Products. Global Veterinaria 11: 685-688.
33. Mohammed FA (2011) The Incidence of *Enterobacteriaceae* Causing Food Poisoning in Some Meat Products. Adv J Food Sci Technol 3: 116-121.
34. Cabedo L, Picart i Barrot L, Teixidó i Canelles A (2008) Prevalence of *Listeria monocytogenes* and *Salmonella* in Ready-To-Eat Food in Catalonia, Spain. J Food Prot 71: 855-859.
35. Banks JG, Board RG (1983) The Incidence and Level of Contamination of British Fresh Sausages and Ingredients with *Salmonellas*. J Hyg (Lond) 90: 213-223.
36. Zaki EMS (2003) Risk Assessment of Ready Prepared Meat Products. PhD Thesis (Meat Hygiene), Fac Vet Med, Cairo university, Egypt.
37. Ahmed AAH, Abd-El-Rahman HA, Moustafa MK (1988) Incidence of *Enterobacteriaceae* in Some Selected Food Stuffs. Assuit Vet Med J 20: 104-109.
38. Vazgecer B, Ulu H, Oztan A (2004) Microbiological and Chemical Qualities of Chicken Doner Kebab Retailed on the Turkish Restaurants. Food Control 15: 261-264.
39. Ismail AH (2008) Effential microbiological quality control points associated with some locally processed meat products. MVSc Thesis, Faculty of Vet Med, Cairo University, Egypt.
40. Shaltout FA (1998) Incidence of Proteolytic and Psychrotrophic Organisms in Some Meat Products. Alex J Vet Science 14: 97-107.
41. Roberts TA, Britton CR, Hudson WR (1980) The Bacteriological Quality of Minced Beef in the UK. J Hyg Lond 85: 211-217.
42. Abd El-Atty NS, Meshref AMS (2007) Prevalence o*f Salmonella* and *E. coli* O157 in Some Foods. 5th Scientific Conference, Beni-Suef Vet Med J 73-78.
43. Molla B, Kleer J, Sinell H (1994) Detection of *Salmonella* in Food by Immuno-magnetic Separation. Archiv Fur Lebensmittelhgiene 45: 112-113.
44. Ejeta G, Molla B, Alemayehu D, Muckle A (2004) *Salmonella* Serotypes Isolated from Minced Meat Beef, Mutton and Pork in Addis Ababa, Ethiopia. Revue Med Vet 155: 547-551.
45. Mahmood NR, Awni DH, Dhaher FH, Jameel MM, HS Rasheed (2011) Isolation and Diagnosis of *Salmonella* in Animal Origin Food, Import Feed in Baghdad Local Markets and Local Poultry Farms. Iraq Acad Sci J 3: 1-19.
46. Elsayed MS, Abdeen E, Akiela MA, Farouk T, Zahran RN (2014) Real Time and Conventional PCR for Characterization of *Salmonella* sp. from Imported Meat to Egypt. Adv Anim Vet Sci 2: 199-203.
47. Libby JA, Brandly JP (1975) Meat Hygiene. 4th Edition LEA and Febiger, Cornell University, Philadelphia, USA.
48. Escartin EF, Castillo A, Hinojosa-Puga A, Saldaña-Lozano J (1999) Prevalence of *Salmonella* in Chorizo and its Survival Under Different Storage Temperatures. Food microbiol 16: 479-486.
49. Field RA, Smith FC, Denane DD, Thomas GM, Kotula AW (1977) Sources of Variation at the Retail Level in Bacteriological Condition of Ground Beef. J Food prot 40: 385-388.
50. Sen U, Garode AM (2016) Identification and Detection of *Salmonella* Risk Assessment from Frozen Buffalo Meat Exported from India. J Bacteriol Mycol Open Access 2: 1-4.

51. Rao NM, Nandy SC (1977) Organisms of *Enterobacteriaceae* Family Associated with Animal By-products. Indian J Anim Sci 47: 344-348.

52. Nagappa K, Tamuly S, Brajmadhuri, Saxena MK, Singh SP (2007) Isolation of *Salmonella typhimurium* from Poultry Eggs and Meat of Tarai Region of Uttaranchal. Indian J Biotechnol 6: 407-409.

53. Moustafa NY, Abd El-Hafiz RM, ElBahy EF (2016) Incidence of *Staphylococcus aureus* and *Salmonella* in Poultry Meat. Global Veterinaria 16: 570-578.

54. Darwin KH, Miller VL (1999) Molecular basis of the interaction of *Salmonella* with the intestinal mucosa. Clin Microbiol Rev 12: 405-428.

55. Nucera DM, Maddox CW, Hoien-Dalen P, Weigel RM (2006) Comparison of API 20E and *invA* PCR for Identification of *Salmonella enterica* isolates from Swine Production Units. J Clin Microbiol 44: 3388-3390.

56. Craciunas C, Keul AL, Flonta M, Cristea M (2012) DNA-based diagnostic tests for *Salmonella* strains targeting hilA, agfA, spvC and sef genes. J Environ Manage 95: S15-S18.

57. Whyte P, Mc Gill K, Collins JD, Gormley E (2002) The Prevalence and PCR Detection of *Salmonella* Contamination in Raw Poultry. Vet Microbiol 89: 53-60.

58. Malorny B, Hoorfar J, Hugas M, Heuvelink A, Fach P, et al. (2003) Interlaboratory Diagnostic Accuracy of a *Salmonella* Specific PCR-based Method. Int J Food Microbiol 89: 241-249.