Detection of food borne pathogens from retail chicken

Heba Badr*, Nayera M. AlAtfeehy and Soad A. Nasef

Reference Laboratory for Veterinary Quality Control on Poultry production, Animal Health Research Institute, Ministry of Agriculture, Nadi El-Seid Street, Dokki P.O. Box 246, Giza 12618, Egypt.
*Corresponding author E-Mail: drheba_badr@yahoo.com

A B S T R A C T

Food borne pathogens are a serious public health problem. Poultry are often associated with food borne disease outbreaks. The objective of this study was to investigate the distribution of food borne pathogens associated with manipulation of chicken meat contaminated with *Salmonella* spp., *E. coli*, *Staphylococcus aureus,* C*ampylobacter* spp. and *Listeria monocytogenes*. 104 retail chicken meat samples were examined (51 imported frozen chicken meats and 53 local chicken meats). *Salmonella* was detected in the percentage of 3.8% (4/104), 1 isolate was *S. Kentucky* and 3 were *S. Magherafelt*. *E. coli* were isolated with percentage of 35.6% (37/104) with different serotypes. On the other hands isolation of *S. aureus* was 27.9% (29/104) revealed from 8 local chicken's samples and 21 imported frozen chicken's samples. While *Campylobacter* appeared with percentage reached to 4.8% (5/104) after confirmation with PCR, which identified *Campylobacter coli*. There is no record for *Listeria Monocytogens*, but *Listeria spp*. was present with percentage of 26.9% (28/104). The identification of typical colonies revealed L. *Ivanovi* and *L. Welshimeri*.

Key words: *Chicken meats, Salmonella, Listeria monocytogen, Campylobacter, Staphylococcus aureus, E. coli*

1. INTRODUCTION

Contamination of poultry meat with food borne pathogens remains an important public health issue and adds significantly to the cost of food production and healthcare. It is also a possible cause of mortality. Numerically, the most important agents are *Salmonellae* and *Campylobacter spp.* (Cavitte, 2003). *Salmonella* normally is found in poultry, which indicated as an important vehicle in food borne diseases inducing salmonellosis as one of the most frequently reported food borne diseases worldwide (WHO/ FAO, 2002). *Salmonella* and *Campylobacters* survive in the alimentary tract of warm blooded animals. Outside, only *Salmonella* could survive in environment, while Campylobacter appear less well adapted as it needs to grow in high moisture, low oxygen and high temperature than 30ºC. Also, Campylobacter are sensitive to drying, freezing and thawing which affect on its rate of isolation from poultry meat (Mbata, 2005). On the other hands, *Escherichia coli* are an indicator of fecal contamination in poultry meats. Some strains of *E. coli* are highly pathogenic in human and animal. People with low immunity are the prime target of the pathogenic strains of *E.coli* (Akbar and Anal, 2011). Also, *Staphylococcus aureus* is considered

one of the most important staphylococci species and the third worldwide cause of the food borne pathogens reported cases (Tamarapu et al., 2001). The presence of any species of *Listeria* in food is an indicator of poor hygiene. Listeriosis arises from food contamination with *Listeria* causing encephalitis, abortion, and septicemia (Dhanashree et al., 2003). The incidence of *Listeria* in meats can be attributed either to fecal contamination during evisceration, or to the actions of food handlers (Fenlon et al., 1996). The genus *Listeria* currently has 8 recognized species including *L. monocytogenes, L. ivanovii, L. seeligeri, L. innocua, L. welshimeri, L. grayi* (Johnson et al., 2004), *L. marthii* (Graves et al., 2010), and *L. rocourtiae* (Leclercq et al., 2010). Only *L. monocytogenes* and *L. ivanovii* are pathogenic and cause listeriosis (Robinson et al., 2000). *L. monocytogenes* infects both humans and animals, while *L. ivanovii* is principally an animal pathogen that rarely occurs in humans (Low and Donachie, 1997).

Therefore, the present study was undertaken to throw the light on some food born bacteria as *Salmonella, E. coli, Staph. aureus, Campylobacter spp. And Listeria Monocytogenes* isolated from

chicken meat and their public health importance and to determine the level of contamination in different chicken meats sample.

2. MATERIALS AND METHODS

2.1. Samples collection:

104 retail meat samples were collected from 51 imported frozen chicken and 53 local slaughtered chicken from different Cairo and Giza supermarkets and storage facilities from 2014 to 2015. Samples were transported to the laboratory in cold ice box for bacterial examination as soon as possible.

2.2. Bacterial isolation and identification:

All samples were examined bacteriologically for presence of *Salmonella*, *E .coli, S. aureus, Listeria monocytogens* and *Campylobacter Spp.* Isolation and identification of *Salmonella*, *E .coli*, coagulase positive *Staphylococci*, *Listeria monocytogens* and *Campylobacter Spp.* were done according to standard methods (BAM, 2001; International organization for standardization (ISO), 2002; ISO, 2004, 2006; Lee and Nolan, 2008) respectively.

Serological identification of *Salmonella* was done according to ISO (2014) for the determination of Somatic (O) and Flagellar (H) antigens using Salmonellae antiserum (DENKA SEIKEN Co., Japan) and (SIFIN Co., Germany) and serological typing of Somatic (O) antigens of *E.coli* was carried out according to Lee et al. (2009) using (DENKA SEIKEN Co., Japan) antiserum. Api for

Listeria (BioMérieux®, France) for identification of *Listeria* strains which have the same characteristic shape as *L. monocytogens* on ALOA agar

2.3. Conventional PCR technique:

2.3.1. Extraction:

DNA of enriched samples was extracted using commercially available kit, QIAamp DNA Mini Kit, Catalogue no.51304.

2.3.2. PCR amplification:

23S rRNA, *hipO* and *glyA* genes were amplified according to refernces mentioned in Table (1). Primers were utilized in a 25μl reaction containing 12.5 μl of EmeraldAmp Max PCR Master Mix (Takara, Japan), 1 μl of each primer of 20 pmol concentrations, 4.5 μl of water, and 6 μl of template. The reactions were performed in a Biometra T3 thermal cycler.

2.3.3. Analysis of the PCR Products:

The products of PCR were separated by electrophoresis on 1 % agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 15 μl of the PCR products were loaded in each gel slot. A 100 bp and 100 bp plus DNA Ladder (Qiagen, Germany, GmbH) were used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

Table (1): Design of primers and the size of amplified products required for detecting the tested genes.

Target gene	Primer sequence (5'-3')	Amplicon (bp)	References
Campylobacter 23S rRNA	F: TATACCGGTAAGGAGTGCTGGAG R: ATCAATTAACCTTCGAGCACCG	650 bp	
C. jejuni *hipO*	F: ACTTCTTTATTGCTTGCTGC R: GCCACAACAAGTAAAGAAGC	323 bp	(Wang et al., 2002)
C. coli *glyA*	F: GTAAAACCAAAGCTTATCGTG R: TCCAGCAATGTGTGCAATG	126 bp	

3. RESULTS

Bacteriological examination of 104 retail meat samples (51 imported frozen chickens and 53 local slaughtered chickens) for detection of most food poisoning bacteria in chicken including *Salmonella, E. coli, S. aureus, Campylobacter* and *Listeria Monocytogens* are shown in Table (2). The percentage of *Salmonella* isolation were 3.8% (4/104) detected in 3 samples from local chickens and 1 from imported chickens. Serological identification of positive isolates showed *S. Magherafelt* (O8,20) (H i; l, w) in the 3 local meat

chicken isolates. While isolate of imported chicken sample was *S. Kentucky* (O8,20) (H i; z_6). *E. coli* were isolated with percentage of 35.6% (37/104) resulted from 17 isolates from local chickens and 20 isolates from imported chickens with serological identification appeared different serotypes as shown in Table (3). On the other hands, *S. aureus* isolation percentage was 27.9% (29/104) resulted from 8 local chickens' samples and 21 imported chicken's samples. While *Campylobacter* showed low isolation, percentage reached to 1.9% (1/51). On the other hands, this percentage was changed when the samples from

enriched Bolton broth confirmed using PCR to be 4.8% (5/104) as shown in Photo (1). All positive results were from samples of imported chickens, and identified as *Campylobacter coli* using PCR as shown in Photo (2).

No record for *Listeria Monocytogens* isolation, instead of it we found 26.9% (28/104) *Listeria spp*. In local chicken samples, 19 isolates and in imported chickens was 9 isolates were positive. Only 3 isolates from imported chicken showed the same character of *L. Monocytogens* in ALOA media, with identification of these 3 isolates as 2 isolates *L. Ivanovi* (identified no. 2660 and 2020) and one isolates *L. Welshimeri* (identified no. 2061) using api for *listeria* as shown in photo (3).

Table (2): The result of bacterial pathogens appeared in examined samples.

Samples	*Salmonella* No. (%)	*E. coli* No. (%)	*S. aureus* No. (%)	*Campylobacter* No. (%)	*Listeria Monocytogens* No. (%)
Local chickens (n= 53)	3 (5.7)	17 (32.1)	8 (15.1)	0 (0)	0 (0)
Imported chickens (n= 51)	1 (1.9)	20 (39.2)	21 (41.2)	5 (9.8)	0 (0)
Total	4 (3.8)	37 (35.6)	29 (27.9)	5 (4.8)	0 (0)

Table (3): Serological identification for *E. coli* isolates.

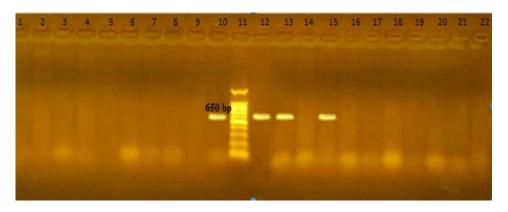| Local chicken meat isolates (No.=17)		Imported frozen chicken (No.= 20)	
Serotypes	No. of isolates	Serotypes	No. of isolates
Poly 2 - O55	2	Poly 2 - O44	1
Poly 2 - O125	1	Poly 2 - O126	1
Poly 4 - O27	1	Poly 2 - O146	1
Poly 5 - O20	2	Poly 3 – O114	1
Poly 6 - O8	2	Poly 3 - O142	1
Poly 6 - O169	1	Poly 4 - O6	1
Poly 8 - O29	2	Poly 5 - O25	1
Poly 8 - O152	1	Poly 6 - O15	5
Poly 8 - O164	1	Poly 6 - O8	2
		Poly 7 - O112 ac	1
Untypable	4	Poly 8 - O152	1
		Untypable	4

Photo (1): molecular identification of Campylobacter spp. using *23S rRNA* gene at 650 bp.
Lane 11: the ladder 100+ (Qiagen).
Lane 10: positive control of campylobacter spp.
Lane 22: negative control.
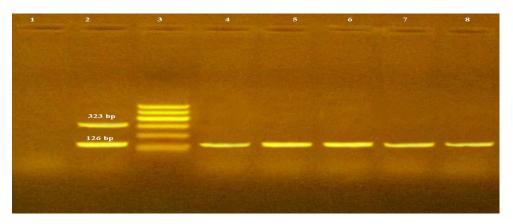Lane 12, 13 and 15: positive pooled samples at 650 bp.

Photo (2): amplification of the *hipO and glyA* genes of *Campylobacter jujeni* and *coli*, positive amplification appeared at 323 bp and 126 bp respectively, lane 1: negative control, lane 2: the positive control C. jujeni and C. coli. lane 3: the ladder 100+ (Qiagen). Lane 4,5, 6, 7 and 8: positive for C. coli

Photo (3): api *Listeria* showed identification no. 2020 of *L. ivanovi*.

4. DISCUSSION

The present study demonstrated that five major pathogenic bacteria were present in retail raw meat products (local and imported) obtained from supermarkets in Cairo and Giza, Egypt and storage facilities. As described by Mulder and Schlundt (1999) who mentioned that the chicken meats could be contaminated with a variety of potentially pathogenic food borne pathogens that may cause human illness such as *Salmonella, Campylobacter, Staphylococcus aureus, Escherichia coli* and *Listeria.*

In the present study, *Salmonella* isolation were 3.8% (4/104) revealed from 3 (5.7%) were *S. Magherafelt* from local chickens and 1(1.9%) were *S. Kentucky* from imported chickens. Similarly, Zhao et al. (2001) who isolated *Salmonella* in 4.2% from 212 chicken meat samples. Other show higher percentage of isolation as 8.3% of 60 poultry carcasses by Shanmugasamy et al. (2011), in Ethiopia the study conducted by Todd (1999) showed the incidence of *Salmonella* spp. contamination in retail chicken to be 13.3%. Also, Molla and Mesfin (2003) reported *Salmonella* in chicken meat (15.4%), which identified as *S. Braenderup,* which was the most frequent followed by *S. Typhimurium*. Dhaher et al. (2011) reported rate of 24.76% and Alali et al. (2012) with

prevalence 27% in chicken meat in Russia Federation. Adeyanju and Ishola (2014) obtained 32.1% (17/53) *Salmonella* contamination from retail chicken samples. While, Abdellah et al. (2009) reported *Salmonella* in chicken meat and giblets, 4 different serotypes were identified the *S. Typhimurium* (40.35%) that was the most frequent. In Dakar Cardinale et al. (2003) examined 300 retail chicken carcasses for prevalence of *Salmonella* which revealed 96 (32%) were positive. The most prominent *Salmonella* serovars were *S. Hadar* (40) and *S. Brancaster* (20) while, *S. Kentukey* were (8) isolates. Other authors as Yassin and El-Gammal (2016) isolated *Salmonella* spp. with a percentage of 18%. Furthermore, the serological identification of the obtained isolates revealed the presence of *S. Typhimurium* (6%), *S. Enteritidis* (4%), *S.* Kentucky (4%), *S. Molade* (2%) and *S. Infants* (2%).

E. coli were isolated with percentage of 35.6% (37/104) from 17 local chicken's meat and 20 from imported frozen chickens with serological identification appeared different serotypes. Near to this percentage Zhao et al. (2001) isolated *E. coli* from contaminated carcasses with percentage (38.7%). While Adeyanju and Ishola (2014) reported 47.2% (25/53) *E. coli* isolation. While, in Kafr elshiekh Governorate Yassin and El-Gammal (2016) detected *E. coli* in 12% of 50 examined

samples, and the serological identification of the obtained isolates revealed the presence of the following serotypes O78, O103:H2, O1:H7 and O125:H21.

S. aureus isolation percentage was 27.9% (29/104) revealed from 8 local chicken's samples and 21 imported frozen chicken's samples. Nearly similar results were detected by Momtaz et al. (2013) who isolated *staph. aureus* at a rate of 22.77%. Higher incidence were obtained by Amin (2008); Kozacinski et al. (2006) and Mohammed (2013) at incidence of 30.03%, 37%, and 32% respectively. While Yassin and El-Gammal (2016) showed 20% (10/50) of the examined samples contained *Staph. aureus*.

Campylobacter showed low isolation percentage reached to 1.9% (1/51), while this percentage was changed when confirmed the samples from enriched Bolton broth using PCR to 4.8% (5/104). All *Campylobacter* spp. were *Campylobacter coli* from imported chicken samples. Similar to our finding, Gritti1 et al. (2011) clarified that the *Campylobacter* is extremely susceptible to a variety of environmental stresses; hence the difficulty to establish cultures of the microorganism in the laboratory. In addition, it has been shown to decline in refrigerated and frozen foods. So, the chilled or frozen poultry meat samples using ISO 10272-1:2006 and confirmed by PCR assay show no incidence of *Campylobacter spp.* Which indicated that the presence rate of *Campylobacter* in chilled and frozen poultry meat samples were lower. In contrary, Zhao et al. (2001) who reported that the chicken was the most frequently contaminated with *Campylobacter* (70.7%). In Dakar retail chicken carcasses were examined by Cardinale et al. (2003) with prevalence of *Campylobacter spp.* from 168/300 (56%) of the samples. *C. jejuni* was more frequently isolated (59%) than *C. coli* (27%).

Listeria spp. isolation was 26.9% (28/104) from local chicken samples 19 isolates and from imported frozen chickens was 9 isolates. While, no record for *Listeria Monocytogens* isolation. Only 3 isolates from imported chicken showed the same character of *L. Monocytogens* in ALOA media, with identification of these 3 isolates appeared 2 isolates *L. Ivanovi* and one isolate was *L. Welshimeri* by api confirmation. Similarly, seven articles met the inclusion criteria; however, no reports of *L. monocytogenes* were obtained (Mercado et al., 2012). Also, Dhanashree et al. (2003) reported the absence of *L. monocytogenes* from raw chicken samples in India and Pelisser et al. (2001) mentioned that the occurrence of *Listeria* species in refrigerated chicken carcasses was occurred in 21/48 (43.7%) samples. While Ismaiel et al. (2014) isolated *listeria spp.* from frozen

chicken meat with percentage 1 (3.33%) identified as *L. innocua* and *L. grayi* in one sample and no *L. monocytogens* were present. Waldroup (1996) found that there was a variation in incidence ranging from 2 to 94% for the isolation of *Listeria* species from chicken depending upon the country of origin and the method employed for isolation.

5. CONCLUSION

Raw retail chicken meats (frozen and chilled) were potential vehicles for transmitting food-borne diseases, and our findings stress the need for increased implementation of hazard analysis of critical control point (HACCP) in local products and examined the imported frozen chickens to provide safety food for consumer.

6. ACKNOWLEDGEMENT

Many thanks to Dr. Ahmed Abdelhalem and Dr. Ahmed M. Erfan Biotechnology unit, Reference Laboratory for Veterinary Quality Control on Poultry production, Animal Health Research Institute, Ministry of Agriculture for its help in PCR practical work.

7. REFERENCES

Abdellah, C., Fouzia, R.F., Abdelkader, C., Rachida, S.B., Mouloud, Z., 2009. Prevalence and anti-microbial susceptibility of Salmonella isolates from chicken carcasses and giblets in Meknes, Morocco. Afr J Microbiol Res 3, 215-219.

Adeyanju, G.T., Ishola, O., 2014. Salmonella and Escherichia coli contamination of poultry meat from a processing plant and retail markets in Ibadan, Oyo State, Nigeria. Springerplus 3, 139.

Akbar, A., Anal, K.A., 2011. Food safety concerns and food- borne pathogens, Salmonella, Escherichia coli and Campylobacter. FUUAST journal of Biology 1, 5-17.

Alali, W.Q., Gaydashov, R., Petrova, E., Panin, A., Tugarinov, O., Kulikovskii, A., Mamleeva, D., Walls, I., Doyle, M.P., 2012. Prevalence of Salmonella on retail chicken meat in Russian Federation. J Food Prot 75, 1469-1473.

Amin, H. 2008. Characterization of certain bacteria isolated from deboned poultry meatPhD, Alex. Univ. Egypt.

BAM, 2001. Bacteriological Analytical Manual, (Food and Drug Administration), 8th Edition Chapter 12 Bennett, R. W. And Lancette, G. A.: "Staphylococcus aureus".

Cardinale, E., Perrier Gros-Claude, J.D., Tall, F., Cissé, M., Guèye, E.F., Salvat, G., 2003. Prevalence of Salmonella and Campylobacter in Retail Chicken Carcasses in Senegal. Revue élev. Méd. vét. Pays trop. 56, 13-16.

Cavitte, J.C., 2003. Present and future control of food-borne pathogens in poultry; revision of the European Community legislation on zoonoses. Proceedings of the XVI European Symposium on the Quality of Poultry Meat. Saint-Brieuc 1, 46-58.

Dhaher, F.H., Awni, M.N., Mahmood, M.M., Jamil, H.S., 2011. Isolation and diagnosis of Salmonella in animal origin food, import feed in Baghdad local markets and local poultry farms. Iraq Acad. Sci. J. 5, 1-19.

Dhanashree, B., Otta, S.K., Karunasagar, I., Goebel, W., Karunasagar, I., 2003. Incidence of Listeria spp. in clinical and food samples in Mangalore, India. Food Microbiology 20, 447-453.

Fenlon, D.R., Wilson, J., Donachie, W., 1996. The incidence and level of Listeria monocytogenes contamination of food sources at primary production and initial processing. Journal of Applied Bacteriology 81, 641-650.

Graves, L.M., Helsel, L.O., Steigerwalt, A.G., Morey, R.E., Daneshvar, M.I., Roof, S.E., Orsi, R.H., Fortes, E.D., Milillo, S.R., den Bakker, H.C., Wiedmann, M., Swaminathan, B., Sauders, B.D., 2010. Listeria marthii sp. nov., isolated from the natural environment, Finger Lakes National Forest. Int J Syst Evol Microbiol 60, 1280-1288.

Gritti1, D., Luiz Vaz, C.S., Voss-Rech, D., Alves, L., Bortolini, F., 2011. Thermophilic Campylobacter Survey in Chilled and Frozen Poultry Meat at Retail in Concŕdia, Santa Catarina. Acta Scientiae Veterinariae 39, 976.

International organization for standardization (ISO), 2002. Microbiology of food and animal feeding stuffs – Horizontal method for the detection of Salmonella. International Standards Organization, Geneva 6579 part 1.

Ismaiel, A.A., Ali, A.E., Enan, G., 2014. Incidence of Listeria in Egyptian Meat and Dairy Samples Food. Sci. Biotechnol. 23, 179-185.

ISO, 2004. Horizontal methods for the detection of Listeria monocytogenes. Geneva ISO 11290-1:1996 Amand. 1.

ISO, 2006. Microbiology of food and animal feeding stuffs – Horizontal method for the detection of Campylobacter spp. International Standards Organization, Geneva. Switzerland 10272 part 1.

ISO, 2014. Microbiology of food and animal feeding stuffs – Horizontal method for the detection of Salmonella. International Standards Organization, Geneva 6579 part 3.

Johnson, J., Jinneman, K., Stelma, G., Smith, B.G., Lye, D., Messer, J., Ulaszek, J., Evsen, L., Gendel, S., Bennett, R.W., Swaminathan, B., Pruckler, J., Steigerwalt, A., Kathariou, S., Yildirim, S., Volokhov, D., Rasooly, A., Chizhikov, V., Wiedmann, M., Fortes, E., Duvall, R.E., Hitchins, A.D., 2004. Natural atypical Listeria innocua strains with Listeria monocytogenes pathogenicity island 1 genes. Appl Environ Microbiol 70, 4256-4266.

Kozacinski, L., Hadziosmanovic, M., Zdolex, N., 2006. Microbiological quality of poultry meat on the Croatian market. Veterinarski Ashiv 764, 305-313.

Leclercq, A., Clermont, D., Bizet, C., Grimont, P.A., Le Fleche-Mateos, A., Roche, S.M., Buchrieser, C., Cadet-Daniel, V., Le Monnier, A., Lecuit, M., Allerberger, F., 2010. Listeria rocourtiae sp. nov. Int J Syst Evol Microbiol 60, 2210-2214.

Lee, G.Y., Jang, H.I., Hwang, I.G., Rhee, M.S., 2009. Prevalence and classification of pathogenic Escherichia coli isolated from fresh beef, poultry, and pork in Korea. Int J Food Microbiol 134, 196-200.

Lee, M.D., Nolan, K.L., 2008. A laboratory manual for the isolation and identification of avian pathogen, 5th ed. American Association of Avian Pathologists.

Low, J.C., Donachie, W., 1997. A review of Listeria monocytogenes and listeriosis. Vet J 153, 9-29.

Mbata, T.I., 2005. Poultry meat pathogens and its Control. Internet Journal of Food Safety 7, 20-28.

Mercado, M., Avila, J., Rey, M., Montoya, M., Gamboa, A., Carrascal, A.K., Correa, D.X., 2012. Brotes por Salmonella spp., Staphylococcus aureus y Listeria monocytogenes asociados al consumo de pollo. Revisión sistemática de la literatura. Biomédica 32, 375-385.

Mohammed, I.A. 2013. Microbiological comparison between broiler chickens produced in poultry abattoir and private shopsPhD, Alex. Univ. .

Molla, B., Mesfin, A., 2003. A survey of Salmonella contamination in chicken carcass and giblets in Central Ethiopia.

Revue De Medecine Veterinaire 154, 267-270.

Momtaz, H., Dehkordi, F.S., Rahimi, E., Asgarifar, A., Momeni, M., 2013. Virulence genes and antimicrobial resistance profiles of Staphylococcus aureus isolated from chicken meat in Isfahan province, Iran. Journal of Applied Poultry Research 22, 913-921.

Mulder, R.W.A.W., Schlundt, J., 1999. Safety of poultry meat: from farm to table. In: International Consultative Group on Food Irradiation (ICGFI), FAO/IAEA/WHO.

Pelisser, M.R., Mendes, S.D.C., Sutherland, A.D., Batista, C.R.V., 2001. Detection of Listeria species in refrigerated chicken carcasses using clearviewtm and a modified conventional culture method. Brazilian Journal of Microbiology 32, 113-116.

Robinson, R.K., Batt, C.A., Patel, P.D., 2000. Encyclopedia of Food Microbiology. Academic Press, San Diego, CA, USA.

Shanmugasamy, M., Velayutham, T., Rajeswar, J., 2011. Inv A gene specific PCR for detection of Salmonella from broilers. Vet. World 4, 562-564.

Tamarapu, S., McKillip, J.L., Drake, M., 2001. Development of a multiplex polymerase chain reaction assay for detection and differentiation of Staphylococcus aureus in dairy products. J Food Prot 64, 664-668.

Todd, C.D., 1999. Food borne and waterborne diseases in developing countries- Africa and the Middle East. Dairy Food Environ Sanitation 21, 110-122.

Waldroup, A.L., 1996. Contamination of raw poultry with pathogens. Worlds Poultry Science Journal 52, 7-25.

Wang, G., Clark, C.G., Taylor, T.M., Pucknell, C., Barton, C., Price, L., Woodward, D.L., Rodgers, F.G., 2002. Colony multiplex PCR assay for identification and differentiation of Campylobacter jejuni, C. coli, C. lari, C. upsaliensis, and C. fetus subsp. fetus. J Clin Microbiol 40, 4744-4747.

WHO/ FAO, 2002. (World Health Organization, Food and Agriculture Organization of the United Nations). Risk assessments of Salmonella in eggs and chicken broilers. Series no. 2 Geneva: 328.

Yassin, S.A., El-Gammal, G.A., 2016. Detection of some pathogenic bacteria in chicken meat sold in poultry shops. Assiut Vet. Med. J. 62, 45-53.

Zhao, C., Ge, B., De Villena, J., Sudler, R., Yeh, E., Zhao, S., White, D.G., Wagner, D., Meng, J., 2001. Prevalence of Campylobacter spp., Escherichia coli, and Salmonella serovars in retail chicken, turkey, pork, and beef from the Greater Washington, D.C., area. Appl Environ Microbiol 67, 5431-5436.