Molecular detection of some virulence genes in multidrug resistant Salmonella species isolated from chicken meat products and raw milk

Ebeed Saleh¹, Hanan El Lawyed², Eman Khedr² and Eman Ali³*

¹Food Hygiene and Control Department, Faculty of Veterinary Medicine, Damanhour University, Egypt
²Food Control Department, Animal Health Research Institute, Zagazig branch, Egypt
³Food Hygiene and Control Department, Faculty of Veterinary Medicine, Damanhour University, Egypt

ABSTRACT

Salmonella still has a serious foodborne outbreak with public health risk. Chicken meat, chicken meat products, and raw milk are important reservoir for Salmonella. In the current study, a total of 120 samples of chicken meat and chicken meat products (breast, thigh, giblets, frozen thigh, nuggets, burger, shish and luncheon, 15 of each) and 50 raw cow milk samples were tested for prevalence, serotyping, virulence genes, and antibiotic resistance profiles of Salmonella spp.. The prevalence of Salmonella spp. was 11.67% in chicken meat samples with the following incidence on each sample group: 13.33, 20, 26.67, 0, 6.67, 6.67, 13.33 and 6.67%, respectively. Raw milk samples overall occurrence of Salmonella spp. was 6%. Serological identification of the isolated Salmonella revealed presence of five different serotypes including S. kentukay, S. entritides, S. typhimurium, S. lindenberg and S. bassa. All isolated Salmonella spp. harbored stn gene; while S. kentukay, S. entritides and S. typhimurium harbored mgtC gene but invA was found in S. entritides, S. typhimurium, S. lindenberg and S. bassa. Also, sopB was detected in S. kentukay, S. entritides, S. typhimurium and S. lindenberg.

The isolated Salmonella spp. was resistant to sulphamethoxazol trimethoprin, chloramphenicol, and penicillin (100%). Meanwhile, the sensitivity was 70.6% % to ampicillin, enrofloxacin and amoxycillin clavulanic. The results confirm the importance of application of strict hygienic measures in food industry and proper use of antibiotics for meat and milk producing animals.

Keywords: Chicken meat, Raw milk, Salmonella spp., Virulence genes, Antibiotic resistance

1. Introduction

Despite the high nutritional value of chicken meat and milk, they could be incriminated with many health hazards and food poisoning outbreaks to the consumers. Chicken meat and raw milk are associated with Salmonella outbreaks around the world (CDC, 2018). Chicken meat and its products can be contaminated from different sources starting from defeathering, evisceration, and the subsequent during processing in plant (Houf et al., 2002; Yar et al., 2020). Raw milk could be contaminated with Salmonella from feces of infected dairy animal and milkers, infected udder, milking equipment, air, and animal insects (Ponce et al., 2008).

The Centre for Disease Prevention and Control (CDC, 2021) reported in the United States about 1.35 million infections with 26,500 hospitalizations, and 420 deaths by Salmonella every year and food are the main source most of these illnesses.

*Corresponding author: Dr. Eman Ali
E-mail address: emanhandy03@vetmed.dmu.edu.eg
Food Hygiene and Control Department, Faculty of Veterinary Medicine, Damanhour University, El-Beheira, Egypt

PISSN: 2636-3003 EISSN: 2636-2996

The clinical symptoms of Salmonella infection include typhoid fever, enteritis, and bacteremia (Santos et al., 2001). Non-typhoid Salmonellosis has been linked with acute gastroenteritis with unpleasant effects on the surrounding organs (Su et al., 2004).

Salmonella Pathogenicity Island 1 (SPI1) is found in all Salmonella spp., it is a genetic element on the chromosome which contains the virulence genes, encoding for factors responsible for invasion of the epithelium cells (Hensel, 2004) as well as adhesions, intracellular survival, antimicrobial resistance, systemic infections, toxin production, and iron and magnesium uptake (Aydin et al., 2011).

The continuous and uncontrolled usage of antimicrobials during livestock production had led to the development of the drug resistance phenomenon among the originated foodborne pathogens including Salmonella (Darwish et al., 2013). The current study was conducted to evaluate the level of contamination with multidrug resistant Salmonella spp. contained virulence genes in chicken products and raw milk.

2. Materials and Methods

2.1. Collection of samples

A total of 120 chicken meat products (breast, thigh, giblets, frozen thigh, nuggets, burger, shish and luncheon, 15 of each) and 50 raw cow milk samples were randomly collected from different local groceries and street vendors at Zagazig, Sharkia Governorate and Damanhour, El-Behira Governorate, Egypt, respectively. The samples were aseptically transferred as soon as possible in an ice box to laboratory to be examined bacteriologically.

2.2. Isolation of Salmonella spp. in chicken meat product and raw milk samples (APHA, 2001):

25 g or 25mL of each sample was mixed with 225mL of buffered peptone water and incubated at 37 °C for 18 ± 2 h. Pre-enrichment of 1 mL of previously incubated homogenate was transferred to 10 mL of Rappaport Vassiliadis with soya (RVS broth) and incubated at 41.5 C±1°C for 24 ± 3 h (Vassiliadis et al., 1978). Then, a loopful was streaked on the plates of (XLD) agar, incubated at 37°C±1°C for 24 ± 3 hrs. Morphologically typical colonies (pink to red colonies with black center) were recorded, picked up and were identified biochemically according to Quinn et al., (2002).

2.3. Serological identification of Salmonella:

Determination of both Somatic (O) and flagellar (H) antigens was done by Salmonella antisera (DENKA SEIKEN Co., Japan) according to Kauffman (1974).

2.4. Molecular identification of Salmonella virulence genes:

DNA extraction was conducted by QIA amp Kit according to the manufacturing instructions. Oligonucleotide primer sequences were illustrated in (Table 1). PCR assay was done according to Sambrook et al. (1989).

2.5. Antibiogram of the isolated Salmonella spp.

Antimicrobial susceptibility was tested by the single diffusion assay according to NCCLS (2001). The tested strains were evaluated as susceptible, intermediate, and resistant. Multiple drug resistance (MDR) index for each strain was determined according to Singh et al. (2010). MDR index= Number of resistance (Isolates classified as intermediate were considered sensitive for MDR index) / total Number of tested antibiotics.
The results revealed that these detections were by Jayarao et al. (2006) (6%); husbandry practices (Oliver et al., 2005). The methods, geographical distribution, seasonal variation, and farm associated with different source of samples, sampling standard.

Tesfaw et al. (2013) (1.6%) and different levels of sanitation. This may be due to different localities of isolation either cities or shops which have practices applied during the production process. Also, this variation may this process. In the current study 6% of examined raw milk samples were incompatible with Egyptian standard. The variable incidence rate of Salmonella in raw milk could be associated with different source of samples, sampling and isolation methods, geographical distribution, seasonal variation, and farm husbandry practices (Oliver et al., 2005).

Serological identification of the isolated Salmonella strains revealed that the chicken meat products were contaminated by five different serotypes including S. kentukay, S. enteritides, S. typhimurium, S. lindenberg and S. bassa with a prevalence of 6(42.86%), 4(28.57%), 2(14.29%), 1(7.14%) and 1(7.14%), respectively (Table 2). S. enteritides (4%) and S. typhimurium (2%) were serologically identified in raw milk samples (Table 3).

### Table 1: Oligonucleotide primers sequences for Salmonella spp. PCR

| Gene | Primer Sequence (5’-3’) | PCR conditions | PCR product | Reference |
|------|-------------------------|----------------|-------------|-----------|
| sopB | TCA GAA GRC GTC TAA CCA CTC TAC CGT CCT CAT GCA CAC TC | 94°C 10 min, 94°C 45 sec, 49°C 45 sec, 72°C 45 sec for 35 cycles. | 517 bp | Huehn et al. (2010) |
| mgtC | TGA CTA TCA ATG CTC CAG TGA AT ATT TAC TGG CCG CTA TGC TGT TG | 94°C 5 min, 94°C 30 sec, 58°C 45 sec, 72°C 45 sec for 35 cycles. | 677 bp | Huehn et al. (2010) |
| Stn | CTT TGG TCG TAA AAT AAG GCC TGC CCA AAG CAG AGA GAT TG | 95°C 15 min, 94°C 1 min, 55°C 1 min, 72°C 1 min for 35 cycles. | 260 bp | Makino et al. (1999) |
| invA | GTG AAA TTA TGG CCA GTG GCC AA TCA TCG CAC GTG CAA AGG AAC C | 94°C 1 min, 94°C 60 sec, 64°C 30 sec, 72°C 30 sec for 35 cycles. | 248 | Kumar et al. (2008) |

| Samples (n=15) | S. lindenberg | S. kentukay | S. typhimurium | S. bassa | S. enteritides | Total |
|----------------|--------------|-------------|--------------|---------|--------------|-------|
| Breast         | 1            | 0           | 1            | 0       | 0            | 2     |
| Thigh          | 0            | 2           | 0            | 0       | 1            | 3     |
| Giblets        | 0            | 1           | 1            | 0       | 2            | 4     |
| Frozen thigh   | 0            | 0           | 0            | 0       | 0            | 0     |
| Nuggets        | 0            | 1           | 0            | 0       | 0            | 1     |
| Burger         | 0            | 1           | 0            | 0       | 0            | 1     |
| Shish          | 0            | 1           | 0            | 0       | 1            | 2     |
| Luncheon       | 0            | 0           | 0            | 1       | 0            | 1     |
| Total          | 1(7.14%)     | 6(42.86%)   | 2(14.29%)    | 1(7.14%)| 4(28.57%)    | 14(11.67%) |

| Samples | S. enteritides | S. typhimurium | Total |
|---------|----------------|----------------|-------|
| Raw milk | 2/50 (4 %) | 1/50 (2 %) | 3/50 (6 %) |

### Table 2: Prevalence and serological identification of isolated Salmonella in chicken meat products

### Table 3: Prevalence and serological identification of isolated Salmonella in raw milk

2.6. Statistical analysis:
Statistical analysis of data was done by using the statistical package for social sciences (SPSS-16; Chicago, IL, USA) software and one way analysis of variance (ANOVA).

3. Results and Discussion

3.1. Isolation and identification of Salmonella spp.

Salmonella is an important microorganism which most frequently associated with food-born outbreaks. As illustrated in Table (2,3), the prevalence of Salmonella spp. in the examined chicken meat products and raw milk was 14(120(11.67%) and 3(30(6%), respectively. Salmonella was detected in 2(13.33%), 3(20%), 4(26.67%), 0, 1(6.67%), 1(6.67%), 2(13.33%) and 16.67% of the examined chicken breast, thigh, giblets, frozen thigh, giblets, burger, shish and luncheon, respectively. The highest incidence of Salmonella spp. was in chicken giblets, while Salmonella spp. failed to be detected in frozen chicken thigh samples. The obtained results nearly agree with Nawar (2007) and Ruban and Fairoze (2011) who isolated Salmonella spp. from 11.11 and 71.43 % of chicken thigh, respectively; Rady et al. (2011) who isolated Salmonella spp. from 16% of chicken breast; Samah et al. (2012) who isolated Salmonella spp. from 8% of nuggets and Hassanin et al. (2017) who isolated Salmonella spp. from 30% of giblets. Meanwhile, Salmonella spp. not detected in chicken meat products (Gad, 2004; Khalifa and Abd El- Shaheed 2005).

According to Egyptian Organization for Standardization for Standardization and Quality control (EOS 154 2005) chicken meat and chicken meat products should be free from Salmonella spp. The results revealed that 14(11.67%) of examined chicken meat and its products were incompatible with Egyptian standard. Only, examined frozen chicken thigh samples were compatible this standard. The variation of results may attribute to the differences in manufacturing, handling and the effectiveness of hygienic practices applied during the production process. Also, this variation may be due to different localities of isolation either cities or shops which have different levels of sanitation.

For raw milk samples, previous studies reported variable prevalence of Salmonella in raw milk as determined by Jayarao et al. (2006) (6%); Tesfaw et al. (2013) (1.6%) and Omar et al. (2018) (52%). While other studies did not report Salmonella from raw milk samples (Mhone et al., 2012; Zeinhom and Abdel-Latef, 2014; Elafify et al., 2019). EOS 154-1(2005) stated the raw milk must be free from Salmonella. In the current study 6% of examined raw milk samples were incompatible with Egyptian standard. The variable incidence rate of Salmonella in raw milk could be associated with different source of samples, sampling and isolation methods, geographical distribution, seasonal variation, and farm husbandry practices (Oliver et al., 2005).

Serological identification of the isolated Salmonella strains revealed that the chicken meat products were contaminated by five different serotypes including S. kentukay, S. enteritides, S. typhimurium, S. lindenberg and S. bassa with a prevalence of 4(28.57%), 2(14.29%), 1(7.14%) and 1(7.14%), respectively (Table 2). S. enteritides (4%) and S. typhimurium (2%) were serologically identified in raw milk samples (Table 3).
Table 4: Distribution of virulence genes among Salmonella serotypes

| Serotypes  | Stn | sopB | mgTC | InvA |
|-----------|-----|------|------|------|
| S. enterikay | +ve | +ve | +ve | -ve |
| S. enteritides | +ve | +ve | +ve | +ve |
| S. bassa | +ve | -ve | -ve | +ve |
| S. linenberk | +ve | +ve | -ve | +ve |
| S. typhimuriam | +ve | +ve | +ve | +ve |

As recorded in Table (5); hundred percent of the isolated Salmonella spp. was resistant to chloramphenicol, sulphonamethoxazol trimethoprim, and penicillin. Furthermore, the resistance was 88.2% to oxytetracyclin and kanamycin, and 64.7% to cefadroxil and doxycyclin. Meanwhile, the sensitivity was 70.6% to ampicillin, enrofloxacin and amoxycillin clavulanic acid but it was 58.8% and 47% to gentamicin and neomycin, respectively with different MDR value for each isolate (Table 5,6). Saad et al. (2015) and Almashhadany, (2019) were reported similar resistance rate of Salmonella. Antibiotic resistant of Salmonella is linked with the misuse of antimicrobial agents for food producing animals; Salmonella resistant strains can be transmitted to consumers through food (Nygard et al., 2008) constituting public health hazards and affects the efficacy of drug treatment in humans (Abdellah et al., 2009).

Table 5: Antimicrobial susceptibility of the isolated Salmonella spp.

| Antimicrobial agent | Sensitive | Intermediate | Resistant |
|--------------------|-----------|--------------|----------|
| Chloramphenicol     | 0         | 0            | 17 100   |
| Cefadroxil          | 0         | 0            | 17 100   |
| Sulphamethoxazol    | 0         | 0            | 17 100   |
| Trimethoprim        | 0         | 0            | 17 100   |
| Kanamycin           | 2         | 11.7         | 0 15     |
| Dofyccyclin         | 6         | 35.3         | 0 11     |
| Oxytetracyclin      | 2         | 11.8         | 0 15     |
| Gentamicin          | 10        | 58.8         | 0 7      |
| Penicillin          | 0         | 0            | 17 100   |
| Enrofloxacin        | 12        | 70.6         | 0 5      |
| Amoxycillin Clavulamic acid | 12 | 70.6 | 0 3 | 17.6 |
| Neomycin            | 8         | 47           | 6 35.3   |
| Ampicillin          | 12        | 70.6         | 3 17.6   | 0 0 |

Table 6: Multiple drug resistance (MDR) index of the isolated Salmonella spp.

| Salmonella spp. | Antimicrobial resistance profile | MDR index |
|-----------------|----------------------------------|-----------|
| S. typhimurium  | C, CFR, SXT, K, DO, T, CN, P, ENR, AMC | 0.83 |
| S. enteritides  | C, CFR, SXT, K, DO, T, CN, P, ENR | 0.75 |
| S. enteritides  | C, CFR, SXT, K, DO, T, CN, P, AMC | 0.67 |
| S. enteritides  | C, CFR, SXT, K, DO, T, P | 0.58 |
| S. kentucky     | C, CFR, SXT, K, DO, T | 0.58 |
| S. kentucky     | C, CFR, SXT, K, T, P | 0.50 |
| S. lindenberg   | C, CFR, SXT, T, K | 0.50 |
| S. enteritides  | C, CFR, SXT, K, T, P | 0.50 |
| S. enteritides  | C, CFR, SXT, K, DO, P | 0.42 |
| S. enteritides  | C, CFR, SXT, K, T | 0.42 |
| S. enteritides  | C, CFR, SXT, T, P | 0.33 |
| S. bassa        | C, CFR, SXT, T, P | 0.33 |
| S. typhimurium  | C, CFR, SXT, K, DO, T, CN, P, ENR, AMC | 0.92 |
| S. enteritides  | C, CFR, SXT, K, DO, T, CN, P, ENR, N | 0.83 |
| S. enteritides  | C, CFR, SXT, K, DO, T, CN, P, ENR | 0.75 |

Conclusion

Chicken meat products and raw milk samples were contaminated with Salmonella spp., that harbored virulence genes with multi drug resistant pathogens. Lack of hygiene in handling and production process, inadequate storage and post-process contamination would be the main causes of this contamination. Implementation of hazard analysis and critical control point system (HACCP) as well as food safety and inspection service (FSIS) in all meat and dairy processing units is effective for controlling food poisoning bacteria. Customers should provide adequate heat treatment of chicken products and raw milk to kill Salmonella spp. with proper refrigeration during storage.

Conflict of interests

The authors have not declared any conflict of interests.

Acknowledgement

We would like to thank all staff members of Food Hygiene and Control Department, Faculty of Veterinary Medicine, Damanhour University, Food and Feed Safety Labotary and Animal Health Research Institute, Zagazig Branch.

References

Abdellah, C., Filali Fouzia, R., Abdelkader, C., Bencheikh Rachida, S., Moulood, Z. 2009. Prevalence and anti-microbial susceptibility of Salmonella isolates from chicken carcasses and giblets in Meknès, Morocco. Afr. J. Microbiol. Res., 3(5), 215-219.
Almashhadany, D.A., 2019. Occurrence and antimicrobial susceptibility of Salmonella spp. isolated from visits meat sold at retail outlets in Erbil City, Kurdistan region, Iraq. Ital. J. Food Saf. 8(2), 8233.
APHA (American Public Health Association), 2001. Compendium of Methods for the Microbiological Examination of Foods Fourth edition. F.P. Downes and K. Ito (editors), American Public Health Association, Washington, D.C.
Aydin, A., Muratoglu, K., Sudagidan, M., Bostan, K., Okulu, B. and Harsa, S., 2011. Prevalence and antibiotic resistance of food borne Staphylococcus aureus isolates in Turkey. Food borne Path. Dis. 8(1), 63-69.
Bourgeois, F.S., Wang, L., Rabino, A.F., Everitt, J., Alvarez, M.I., Awadia, S., Wittchen, E., Garcia-Mata, R., Ko, D., 2021. ARHGEF26 enhances Salmonella invasion and inflammation in cells and mice. PLoS Pathog. 10, 1371.
Center for Disease Control and Prevention (CDC). Salmonella. Atlanta, GA: CDC; 2018 (Accessed 2019 Sept 7). Available from: http://www.cdc.gov/salmonella/general/technical.html.
CDC. Salmonella Infections 2021. Available online: https://www.cdc.gov/salmonella/index.html (accessed on December 8, 2021).
Darwish, W.S., Eldaly, E.A., El-Abbasy, M.T., Ikenaka, Y., Nakayama, S. and Ishizuka, M., 2013. Antibiotic residues in food: the African scenario. Jpn. J. Vet. Res. 61, S13-S22.
Ekwanzala, M.D., Abia A.L.K., Keshri J., Momba M.N.B., 2017. Genetic characterization of Salmonella and Shigella spp. isolates recovered from water and riverbed sediment of the Apies River, South Africa. Water SA. 43(3), 387-397.
Elalfify, M., Darwish, W.S., Al-Ashmawy, M., Elsherbini, M., Koseki, S., Kawamura, S. and Abdelhakel, A., 2019. Prevalence of Salmonella spp. in Egyptian dairy products: molecular, antimicrobial profiles and a reduction trial using d-tryptophan. J. Consumer Protect. Food Safety 14(4), 399-407.
Elkenany, R., Elsayed, M., Zakaria, A.I., El-sayed S.A., Rizk, M.A., 2019. Antimicrobial resistance profiles and virulence genotyping of Salmonella enterica serovars recovered from broiler chickens and chicken carcasses in Egypt. BMC Vet. Res. 15, 124.
EOS, 154-12/2005 Egyptian Organization for Standardization and Quality control. Raw milk the Part 1ES:154-1/2005.
EOS., 1651, 2005 Egyptian Organization for Standardization and Quality for Chilled poultry and rabbit (i) and (ii) 1696 for heat treated products. Gad, M.A. 2004. Microbiological evaluation of poultry meat and its products. M.V.Sc., Thesis, Fac. Vet. Med., (Meat hygiene) Sadat branch Minofiya Univ.
Goodman, L.B., McDonough, P.L., Anderson, R.R., Franklin-Guild, R.J., Ryan, J.R., Perkins, G.A., Thachil, A.J., Glaser, A.L., Thompson, B.S., Wang, L., Rabino, A.F., Everitt, J., Alvarez, M.I., Awadia, S., Wittchen, E., Garcia-Mata, R., Ko, D., 2021. ArHGEF26 enhances Salmonella invasion and inflammation in cells and mice. PLoS Pathog. 10, 1371.
Hassanin, F.S., Hassan, M.A., Shaltout, F.A., Shawy, N.A. and Abd-Elhameed, G.A. 2017. Bacteriological criteria of chicken giblets. Benha Vet. Med. J., 33(2); 447-456.
Hensel, M., 2004. Evolution of pathogenicity islands of Salmonella enterica. Int. J. Med. Microbiol., 294, 95-102.

26
Houf, K., De zurrtr, L., Van Hoof, j., Vandamme, P. 2002. Occurrence and distribution of *Acreobacter species* in poultry processing. Fac. Vet. Med. Belgium. J. Food Prot. 65(80), 1233-1239.

Hu, Q., Coburn, B., Deng, W., Li, Y., Shi, X., Lan, Q., Wang, B., Coombes, B.K., Finlay, B.B., 2008. *Salmonella enterica* serovar Senftenberg human clinical isolates lacking SPI-1. J. Clin. Microbiol. 46(4), 1330-1336.

Huehn, S., La Ragione, R.M., Anjum, M., Saunders, M., Woodward, M.J., Bunge, C., Helmut, R., Hauser, E., Guerra, B., Beutlich, J., Brisaibois, A., Peters, T., Svensson, L., Madajczak, G., Litrup, E., Imre, A., Herrera-Leon, S., Mevis, D., Newell, D.G., Malorny, B., 2010. Virulotyping and antimicrobial resistance typing of *Salmonella enterica* serovars relevant to human health in Europe. Foodborne Path. Dis. 7, 523-35.

Jayarao, B. M., Donaldson, S. C., Straley, B. A., Sawant, A. A., Hegde, N. V. and Brown, J. L. 2006. A survey of foodborne pathogens in bulk tank milk and raw milk consumption among farm families in Pennsylvania. J. Dairy Sci. 89, 2451-2458.

Kauffman, G. 1974. Kauffmann white scheme. Acta Path. Microbiol. Sci. 61, 38.

Khalifa, E.M.I. and Abd El-Shaheed, Y.T.U., 2005. Bacteriological evaluation of chicken meat and some chicken meat products sold in kafi El-sheikh governate. 4th Int. Sci.Conf., Mansoura.

Kumar, K., Saklani, A.C., Singh, S., Singh, V.P., 2008. Evaluation of specificity for invA gene PCR for detection of *Salmonella* spp. Proceeding of VIIth Annual Conference of Indian Association of Veterinary Public Health Specialists (IAVPHS), 7-9.

Lan, T.T.Q., Gaucher, M.L., Nhan, N.T.M., Letellier, A., Quessy, S., 2018. Distribution of virulence genes among *Salmonella* serotypes isolated from pigs in southern Vietnam. J. Food Prot. 81(9), 1459-1466.

Makino, S., Kurazono, H., Chongsanguam, M., Hayashi, H., Cheun, H., Suzuki, S., Shirahata, T., 1999. Establishment of the PCR system specific to *Salmonella* spp. and its application for the inspection of food and fecal samples. J. Vet. Med. Sci. 61(11), 1245-1247.

Mhone, T. A., Matope, G., 2018. Prevalence of *Salmonella enterica* enteric serovar Weltevreden from dairy products in Addis Ababa, Ethiopia. African J. Microbiol. Res. 7, 5046-5050.

Morrow, K., Pham, T.T., Turchi, B., Fratini, F., Ebani, V.V., Cerri, D., Bertelloni, F., 2020. Characterization of *Salmonella* spp. Isolates from Swine. Virulence Antimicrob. Resist. Animals. 10, 2418.

Mukhtar, T., Tzouvelekis, M., Pateraki, K. and Papaconoran, N. 1978. Isolation of *Salmonella* from minced meat by the use of a new procedure of enrichment.. Zbl. Bakt. Parasit. Infekt. Krankh. 133(1), pp.69-76.

Nayar, A., Ahmad, I., Rao, A.S., Khan, A., 2008. Prevalence of *Salmonella* and *Staphylococcus aureus* in poultry meat and processed poultry products in Mansoura, Egypt. J. Food Prot. 71(1), 145-146.

Nygard, K., Lassen, J., Vold, L., 2008. Outbreak of *Salmonella typhimurium* infections linked to imported roulette. Foodborne Pathog. Dis. 5(2), 165-173.

Omar, D., Al-Asfamawi, M., Ramadan, H. and El-Sherbiny, M., 2018. Occurrence and PCR identification of *Salmonella* spp. from milk and dairy products in Mansoura, Egypt. Int. Food Res. J. 25(1), pp.446-452.

Ponce E, Khan AA, Cheng CM, Summage WC, Cerniglia CE (2008). 2008. Prevalence and characterization of *Salmonella enterica* serovar Weltevreden from imported sea food. Food Microbiol. 25: 29-35.

Quinn, P.J., Markey, B.K., Carter, M.E., Donnelly, W.J. and Leonard, F.C., 2002. Veterinary Microbiology and Microbial Diseases. Black well scientific publications, Oxford, London.

Rady, E.M., Ibrahim, H.A., Samah, I.A., 2011. Enteropathogenic bacteria in some poultry meat products. Alex. J. Vet. Sci. 31(1), 175-180.

Rahman, H., 2006. Prevalence & phenotypic expression of sopB gene among clinical isolates of *Salmonella enterica*. Indian J. Med. Res. 123, 83-88.

Rubin, S.W. and Fairlce, N., 2011. Effect of processing condition on microbiological quality of market poultry meats in Bengalore, Ind. J. Ani. Vet. Adv. 10(2),188-191.

Saad, S.M., Edris, A.M., Hassan, M.A., Edris, S.N., 2015. Antibiotic sensitivity of *Salmonella* species isolated from chicken meat products. Benha Vet. Med. J. 28(2), 141- 146.

Salehi T.Z., Mahtoumiez, M., Saeedzadeh A., 2005. Detection of invA gene in isolated *Salmonella* from broilers by PCR method. Int. J. Poult. Sci. 4(8), 557-559.

Samaha, I.A., Ibrahim, H.A.A., Hamada, M.O., 2012. Isolation of some enteropathogens from retailed poultry meat in Alexandria province. Alex. J. Vet. Sci. 37(1),17-22.

Sambrook, J., Fritsch, E.F., Mentiates, A., 1989. Molecular cloning. A laboratory manual. Cold spring Harbor Laboratory press, New York.

Santos, R.L., Tsolis, R.M., Zhang, S., Ficht, T.A., Baumler, A.J., Adams, L.G. 2001. Salmonella-Induced Cell Death Is Not Required for Enteritis in Calves. Infect. Immun. 69, 4610-4617.

Singh, A., Yadav, S., Singh, S., Bharti, P. 2010. Prevalence of *Salmonella* in chicken eggs collected from poultry farms and marketing channels and their antimicrobial resistance. Food Res. Int. 43: 2027-2030.