Prevalence and Antimicrobial Susceptibility of Salmonella in Rendered Animal Products Used in Poultry Feed in Turkey [1]

Halil Can KUTAY 1 Emek DÜMEN 2 Onur KESER 1 Ayşe Şebnem BİLGİN 1 Sevgi ERGİN 3 Neşe KOCABAĞLI 1

[1] This study was supported by the Research Fund of the University of Istanbul by the project number of 30430/2013
1 Department of Animal Nutrition and Nutritional Diseases, Faculty of Veterinary Medicine, Istanbul University, TR-34320 Avcılar, İstanbul - TURKEY
2 Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Istanbul University, İstanbul, TR-34320 Avcılar, İstanbul - TURKEY
3 Department of Clinical Microbiology, Cerrahpaşa Faculty of Medicine, Istanbul University, TR-34303, İstanbul – TURKEY

Abstract
The increased prevalence of Salmonella contamination in poultry has gained considerable scientific attention during last decades. In this study, a total of 500 samples of rendered animal products (meat meal, meat-bone meal, blood meal, chicken meal and feather meal) were obtained from several feed factories and rendering plants in Turkey and these samples were analyzed for Salmonella spp., Salmonella Enteritidis and Salmonella Typhimurium status. According to the results, 13, 11 and 8 samples obtained from feed factories were determined positive for Salmonella spp., Salmonella Enteritidis and Salmonella Typhimurium respectively. However, all samples obtained from rendering plants were negative. Antibiotic susceptibility profiles of isolates confirmed as positive were determined by using 17 different antibiotics. It was determined that Salmonella spp. and Salmonella Enteritidis serovars were resistant to amikacin, cephazolin and erythromycin, sensitive to amoxicillin, chloramphenicol, flumoquin, phosphomycin, kanamycin, oxytetracycline, spiramycin, streptomycin, tetracycline, tobramycin and vancomycin and moderate sensitive to gentamicin, linkomycin and rifampicin.

Keywords: Salmonella, Poultry, Rendered animal products, Antibiotic, Susceptibility

INTRODUCTION
Rendered products such as meat and bone meal (MBM), meat meal (MM), poultry meal (PM), feather meal (FM), blood meal (BM) and fish meal (FM) are important animal derived feedstuffs for poultry nutrition [1]. In Turkey, rendering by-products have been used intensively in poultry diets because of their quality protein, calcium and utilisable phosphorus ingredients. Meat-bone meal, the most produced rendering product, has been used only in poultry and pig diets because of BSE (Bovine Spongiform Encephalopathy) risk. The use of meat and bone meal for...
MATERIAL and METHODS

Sample Collection

A total of 500 rendering samples (100 meat meal, 100 meat-bone meal, 100 blood meal, 100 chicken meal, 100 feather meal) were collected from rendering plants and feed manufacturers in several provinces in Turkey. All collected samples were transported to Istanbul University, Faculty of Veterinary Medicine Laboratory under cold-chain procedure and stored at +4°C for further analysis.

Isolation and Identification

Pre-enrichment procedure was applied to samples in non-selective medium (buffered peptone water). After homogenization, all samples were incubated at 37°C for 24 h. For selective enrichment, approximately 0.1 mL of each sample was inoculated to selective enrichment medium (Rappaport Vassiliadis Soy Broth) and all tubes were vortexed before incubation. After the incubation period at 42°C for 24 h, transition to brilliant-green phenol-red lactose sucrose agar, a specific solid medium, was done. Due to the suggestions offered by international procedures, a second specific agar (xylose lysine deoxycholate, XLD agar), was also preferred for parallel study. After selective enrichment, parallel transition was done with Standard plate spreading method to both agar. After the incubation of mediums at 37°C for 24 h, chemical tests were applied for identification of typical colonies. Optionally, motility test was also done by using semi indol motility (SIM) agar. A loop of colony from all Salmonella spp. positive samples was transferred parallely to Hektoen Enteric Agar and Bismuth Sulphite Agar. Black “rabbit-eye” colonies with a black zone and metallic sheen surrounding the colony in Bismuth Sulphite Agar were confirmed as Salmonella Typhimurium and bluish-grey/dark-grey color colonies were confirmed as Salmonella Enteritidis.

Serological Identification

Serogrouping of 32 strains, determined as Salmonella spp. by microbiological isolation, were performed by plate agglutination method. According to agglutination tests performed by using “Phase 1” and “Phase 2” antiseraums, it was determined that 11 and 8 strains pertained to serogroup D1 and B, respectively, and they were serotyped as Salmonella Enteritidis and Salmonella Typhimurium, respectively. Also, 13 isolates evaluated as Salmonella spp. have not reacted positively result with available antiserums.

PCR Analysis

The primer sequences used in PCR analysis for Salmonella spp., Salmonella Typhimurium and Salmonella Enteritidis are shown in Table 1. PCR mix was as follows (final 25 μL): 2 μL DNA samples, 2.5 mM MgCl₂, 10 mM Tris–HCl pH 8.0, 5 mM KCl (0.2 mM from each nucleotide), each...
primer (Metabion Inter-national, Martinsried, Germany) 0.8 pmol/mL, 1 U of Taq DNA polymerase (Fermentas, Vilnius, Lithuania). Initial denaturation heat was at 94°C for 5 min. Then the heat treatments, 1 sec at 94°C, 1 sec at 55°C, and 21 sec at 72°C for extension were applied. After 35 cycles, the procedure was completed with 7 min at 72°C heat treatment for last elongation. Amplification products were analyzed in 1.2% (w/v) agarose gel containing 5 μL safe view (Abm, Richmond, Canada).

**Antibiotic Susceptibility Testing**

Antibiotic sensitivities of isolated *Salmonella* strains were determined by disk diffusion method according to Clinical and Laboratory Standards Institute [16]. For testing, bacterial suspensions were prepared according to McFarland 0.5 turbidity degree and 0.1 mL of suspensions were separated to Muller Hinton Agar and then antibiotic disks (amoxicillin, 15 µg; chloramphenicol, 30 µg; flumoxacin, 30 µg; phospho-mycin, 50 µg; kanamycin, 30 µg; oxytetracycline, 30 µg; spiramycin, 100 µg; streptomycin, 10 µg; tetracycline, 30 µg; tobramycin, 30 µg; vancomycin, 30 µg; gentamicin, 10 µg; linkomycin, 10 µg; rifampicin, 5 µg; amikacin, 30 µg; cephalolin, 30 µg; erythromycin, 15 µg) [17] placed on the agar plate. After the incubation of cultures at 37°C for 24 h, diameters of inhibition zones were measured with calliper.

**RESULTS**

In this study, a total of 500 rendering samples were analyzed for *Salmonella* and 32 of samples were positive. While *Salmonella* spp., *Salmonella* Enteritidis and *Salmonella* Typhimurium were determined in 13, 11 and 8 samples of positive samples respectively, all samples obtained from rendering plants were negative. *Salmonella* spp., *Salmonella* Enteritidis and *Salmonella* Typhimurium contamination in rendering samples obtained from several feed factories and rendering plants were presented in Table 2 and protocol numbers of 32 positive samples were listed in Table 3.

Antibiotic susceptibility status for each isolates were also determined by using 17 different antibiotics. It was determined that *Salmonella* spp. and *Salmonella* Enteritidis serovars were resistant to amikacin, cephalolin and erythromycin, sensitive to amoxicillin, chloramphenicol, flumoxacin, phosphomycin, kanamycin, oxytetracycline, spiramycin, streptomycin, tetracycline, tobramycin and vancomycin and moderate sensitive to gentamicin, linkomycin and rifampicin.

Antibiotic susceptibility status for each isolates were also determined by using 17 different antibiotics. It was determined that *Salmonella* spp. and *Salmonella* Enteritidis serovars were resistant to amikacin, cephalolin and erythromycin, sensitive to amoxicillin, chloramphenicol, flumoxacin, phosphomycin, kanamycin, oxytetracycline, spiramycin, streptomycin, tetracycline, tobramycin and vancomycin and moderate sensitive to gentamicin, linkomycin and rifampicin.

Antibiotic susceptibility of strains in 32 positive rendering samples according to their protocol (1-32) numbers were presented in Table 4 and antibiotic susceptibility percentages of *Salmonella* strains isolated from 32 positive rendering samples were presented in Table 5.

**DISCUSSION**

Almost all by-products transported to rendering plants are mostly contaminated with several pathogens. It was reported that pathogene contamination in by-products transported to rendering plants were 23% *E. coli* O157:H7, 50% *Salmonella*, 39% *Cryptosporidium parvum* for cattle origin, 46% *Salmonella*, 49% *Yersinia enterocolitica* for pig origin and 100% *Salmonella* for poultry origin products [18].

*Salmonella* contamination is the most important microbiological threat in rendering process. Although the application of heat under high pressure during the process is very effective in the elimination of agent, the major risk is the continuation of existence of the agent in the facility by cross-contamination, therefore, re-contamination of end-products by primary and secondary factors such as transportation, storage, factory staffs etc. [19].

Troutt et al. [20] reported that plenty of pathogenes such as Salmonella strains, *Listeria monocytogenes, Campylobacter jejuni* and *Clostridium perfringens* contamination were detected in raw materilas obtained in pre-processing stage from 17 rendering enterprises in several states of USA. However, in other study by same researchers it was reported that none of these pathogenes were isolated from processed samples obtained from 9 rendering facilities. According to the results it is estimated that treatment of
Table 2. Salmonella spp., Salmonella Enteritidis and Salmonella Typhimurium contamination in rendering samples obtained from several feed factories and rendering plants

| Samples Obtained from Feed Factories | Occurrence | Incident Rate, % | Isolation and Identification (+)/(-) | PCR Verification (+)/(-) |
|--------------------------------------|------------|------------------|--------------------------------------|--------------------------|
| **Salmonella spp.**                  |            |                  |                                      |                          |
| Meat meal                            | 4/80       | 5                | 4/76                                 | 4/76                     |
| Meat-bone meal                       | 0/80       | 0                | 0/80                                 | 0/80                     |
| Blood meal                           | 3/80       | 3.75             | 3/77                                 | 3/77                     |
| Chicken meal                         | 6/80       | 7.5              | 6/74                                 | 6/74                     |
| Feather meal                         | 0/80       | 0                | 0/80                                 | 0/80                     |
| Total                                | 13/400     | 3.25             | 13/387                               | 13/387                   |
| **Salmonella Enteritidis**           |            |                  |                                      |                          |
| Meat meal                            | 2/80       | 2.5              | 2/78                                 | 2/78                     |
| Meat-bone meal                       | 0/80       | 0                | 0/80                                 | 0/80                     |
| Blood meal                           | 3/80       | 3.75             | 3/77                                 | 3/77                     |
| Chicken meal                         | 6/80       | 7.5              | 6/74                                 | 6/74                     |
| Feather meal                         | 0/80       | 0                | 0/80                                 | 0/80                     |
| Total                                | 11/400     | 2.75             | 11/389                               | 11/389                   |
| **Salmonella Typhimurium**           |            |                  |                                      |                          |
| Meat meal                            | 2/80       | 2.5              | 2/78                                 | 2/78                     |
| Meat-bone meal                       | 0/80       | 0                | 0/80                                 | 0/80                     |
| Blood meal                           | 1/80       | 1.25             | 1/79                                 | 1/79                     |
| Chicken meal                         | 5/80       | 6.25             | 5/75                                 | 5/75                     |
| Feather meal                         | 0/80       | 0                | 0/80                                 | 0/80                     |
| Total                                | 8/400      | 2                | 8/392                                | 8/392                    |

Samples Obtained from Rendering Plants

| Occurrence | Incident Rate, % | Isolation and Identification (+)/(-) | PCR Verification (+)/(-) |
|------------|------------------|--------------------------------------|--------------------------|
| **Salmonella spp.**                  |                  |                                      |                          |
| Meat meal  | 0/20             | 0                                    | 0/20                     |
| Meat-bone meal | 0/20         | 0                                    | 0/20                     |
| Blood meal | 0/20             | 0                                    | 0/20                     |
| Chicken meal | 0/20             | 0                                    | 0/20                     |
| Feather meal | 0/20             | 0                                    | 0/20                     |
| Total      | 0/100            | 0                                    | 0/100                    |
| **Salmonella Enteritidis**           |                  |                                      |                          |
| Meat meal  | 0/20             | 0                                    | 0/20                     |
| Meat-bone meal | 0/20             | 0                                    | 0/20                     |
| Blood meal | 0/20             | 0                                    | 0/20                     |
| Chicken meal | 0/20             | 0                                    | 0/20                     |
| Feather meal | 0/20             | 0                                    | 0/20                     |
| Total      | 0/100            | 0                                    | 0/100                    |
| **Salmonella Typhimurium**           |                  |                                      |                          |
| Meat meal  | 0/20             | 0                                    | 0/20                     |
| Meat-bone meal | 0/20             | 0                                    | 0/20                     |
| Blood meal | 0/20             | 0                                    | 0/20                     |
| Chicken meal | 0/20             | 0                                    | 0/20                     |
| Feather meal | 0/20             | 0                                    | 0/20                     |
| Total      | 0/100            | 0                                    | 0/100                    |
appropriate time-temperature can inactivate large group of food pathogenes during rendering process [20].

In a study carried out by Watkins et al.[21], it was reported that 28 different Salmonella strains were isolated from animal feed products and incidence was 18.5%. Pomeroy et al.[22] collected 980 samples of animal feed products from 22 different states in USA and they isolated 43 Salmonella strains originated from secondary contaminations in 170 samples. In a recent study, a total of 201 feed ingredient samples (122 animal by-products and 79 plant by-products) were collected from rendering plants and oilseed industry and it was reported that Salmonella were present in 22.9% of samples and animal by-products had a significantly higher Salmonella contamination rate (34.4%) than plant by-products [23]. In our study, Salmonella serovars (Salmonella spp., Salmonella Enteritidis and Salmonella Typhimurium) were determined in 32 samples of 500 rendered animal products and source of agents were in accord with Watkins et al.[21] and Pomeroy et al. [22]. In this study, salmonella-positive results were only in the samples obtained from feed factories. This finding was associated with secondary contamination sources such as transportation, factory staff and storage conditions and was not in accordance with Ge et al.[23]. Although the revolutionary improvements in food safety have been occurred during the last 50 years, still in existence of cross-contaminations in rendered animal products for poultry feed are questionable [24,25].

Proper storage conditions of feedstuffs produced for poultry feeding by using rendering procedures must be kept in mind as the most efficient factor for breaking the contamination chain of Salmonella. Sutton et al.[26] reported that Salmonella content decreased to the undetectable

| Table 3. Protocol numbers of 32 positive rendering samples |
| Tablo 3. Otuziki pozitif renderin örneğinin protokol numaraları |

| Protocol Number | Sample       | Protocol Number | Sample       | Protocol Number | Sample       |
|-----------------|--------------|----------------|--------------|----------------|--------------|
| 1               | Meat meal    | 14             | Chicken meal | 25             | Chicken meal |
| 2               | Meat meal    | 15             | Chicken meal | 26             | Chicken meal |
| 3               | Chicken meal | 16             | Chicken meal | 27             | Chicken meal |
| 4               | Chicken meal | 17             | Chicken meal | 28             | Chicken meal |
| 5               | Blood meal   | 18             | Chicken meal | 29             | Chicken meal |
| 6               | Chicken meal | 19             | Chicken meal | 30             | Meat meal    |
| 7               | Chicken meal | 20             | Meat meal    | 31             | Meat meal    |
| 8               | Blood meal   | 21             | Meat meal    | 32             | Blood meal   |
| 9               | Meat meal    | 22             | Blood meal   |                |              |
| 10              | Meat meal    | 23             | Blood meal   |                |              |
| 11              | Blood meal   | 24             | Blood meal   |                |              |
| 12              | Chicken meal |                |              |                |              |
| 13              | Chicken meal |                |              |                |              |

| Table 4. Antibiotic susceptibility of Salmonella strains in 32 positive rendering samples according to their protocol (1-32) numbers |
| Tablo 4. Protokol numaralarına (1-32) göre 32 pozitif rendering örneğinde Salmonella türlerinin antibiyotik duyarlılığı |

| Susceptibility | Amik | Amox | Ceph | Chlo | Eryt | Plum | Phos | Gent | Kana | Link | Oxit | Rifa | Spir | Stre | Tetr | Tobr | Vanc |
|----------------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| R              | 1-13 | -    | 1-15 | 11   | 1-12 | 7-8  | 7-8  | 7-18 | 19   | -    | -    | -    | -    | -    | -    | -    | -    |
| S              | 14-16| 1-32 | 16-17| 1-10 | 1-10 | 9-10 | 1-6  | 19   | -    | 1-32 | 1-32 | 1-32 | 1-32 | 1-32 | 1-32 | 1-32 | 1-32 | 1-32 |
| MS             | -    | -    | -    | -    | -    | -    | 1-6  | 11-16| 19-24| 27-32| -    | -    | 1-32 | 1-32 | -    | -    | -    | -    |

R: Resistant to antibiotic; S: Sensitive to antibiotic; MS: Moderate sensitive to antibiotic; Amik: Amikacin; Amox: Amoxicillin; Ceph: Cephazolin; Chlo: Chloramphenicol; Eryt: Erythromycin; Plum: Flumoquin; Phos: Phosphomycin; Gent: Gentamicin; Kana: Kanamycin; Link: Linkomycin; Oxit: Oxitetracycline; Rifa: Rifampicin; Spir: Spiramycin; Stre: Streptomycin; Tetr: Tetracycline; Tobr: Tobramycin; Vanc: Vancomycine
Prevalence and Antimicrobial ... levels in meat-bone meal samples exposed to 30 cfu/g *Salmonella* contamination when kept under 28°C for 48 h.

Because there has been growing public health concern over the worldwide emergence of antibiotic-resistant strains of a number of pathogenic bacteria, including *Salmonella* during the past few decades [27], the other parameter investigated in this study was the determination of the susceptibility to several antibiotics of *Salmonella* strains (*Salmonella* spp., *Salmonella* Enteritidis and *Salmonella* Typhimurium) isolated from samples of rendered animal products produced for poultry feeding. For this purpose, 17 different antibiotics were used. Medical literatures reported that antibiotic resistances of *Salmonella* strains were variable. The rising of multiple resistance to antibiotics has been making *Salmonella* treatment difficult for last twenty years [28]. It was reported that there were epidemic spread, since 1989, of multiresistant *Salmonella* Typhi [29]. In a study, antibiotic resistance pattern of *Salmonella* spp. from chicken eggs, intestines and environmental samples were investigated and identified serotypes such as *Salmonella* Typhi, *Salmonella* Typhimurium, *Salmonella* Enteritidis, and other serotypes were found 100% sensitive to ceftriazone, ciprofloxacin, cephalexin, gentamycin and chloramphenicol, but strains have shown resistance to co-trimoxazole, nalidixic acid, ampicillin, tetracycline and kanamycin [30]. Yildirim et al.[31] reported that resistance of all of the *Salmonella* spp. isolates from raw chicken carcasses, predominant one included *Salmonella* Typhimurium, to penicillin, oxacillin, clindamycin, vancomycin, erythromycin and ampicillin were 100%, 97%, 97%, 92.6%, 89.7% and 85.2%, respectively, also resistance to tetracycline (67.6%), streptomycin (61.7%), neomycin (55.8%) and cephalothin (52.9%) was observed but a small percentage of isolates demonstrate resistance to gentamicin (14.7%), chloramphenicol (10.2%), cefotaxime (2.9%) and amikacin (2.9%). Similarly, Zarakolu et al.[32] reported that resistance of 87 *Salmonella* Typhimurium isolates to ampicillin, trimethoprim-sulfamethoxazole, chloramphenicol were 56%, 90%, 100% respectively, and were sensitive to ciprofloxacin and ofloxacine. Dallal et al.[33] determined that a high percentage of *Salmonella* isolates from chicken and beef meat samples were resistant to nalidixic acid (82%), tetracycline (69%), trimethoprim (63%) and streptomycin (52%) and 68.5% of isolates were multidrug resistant. Similarly, Yan et al.[34] found that *Salmonella* isolates were frequently resistant to sulfamethoxazole (86.4%), sulfoxmethazole/trimethoprim (48.1%), nalidixic acid (30.9%), tetracycline (19.8%), corboxybenzylpenicillin (17.3%), amoxicillin (17.3%) and ampicillin (16.0%) and multiple resistance was found in 29.6% isolates. In our study, all of the isolated strains were sensitive to amoxicillin and chloramphenicol and were resistant to amikacin, cephalozin and erythromycin. However, isolated *Salmonella* spp. and *Salmonella* Enteritidis serovars were resistant to amikacin, cephalozin and erythromycin, sensitive to amoxicillin, chloramphenicol, flumoxin, phosphomycin, kanamycin, oxytetracycline, spiramycin, streptomycin, tetracycline, tobramycin, vancomycin and moderate sensitive to gentamicin, linkomycin and rifampicin. It was also determined that sensitiveness profiles of isolated *Salmonella* Typhimurium serovars to antibiotics, except for cephalozin, were similar to those

![Table 1](attachment:image.png)

**Table 1. Antibiotic susceptibility percentages of Salmonella strains isolated from 32 positive rendering samples**

| Antibiotics | S. spp. | S. Enteritidis | S. Typhimurium |
|-------------|---------|---------------|---------------|
| Amikacin    | R (100%)| R (72.7%) S (27.3%) | R (75.0%) S (25.0%) |
| Amoxicillin | S (100%)| S (100%)       | S (100%)       |
| Cephalozin  | R (100%)| R (81.8%) S (18.2%) | R (50.0%) S (50.0%) |
| Chloramphenicol | R (7.7%) S (92.3%) | R (18.2%) S (81.8%) | R (12.5%) S (87.5%) |
| Erythromycin | R (76.9%) S (23.1%) | R (81.8%) S (18.2%) | R (75.0%) S (25.0%) |
| Flumoxin    | R (15.4%) S (84.6%) | R (9.1%) S (90.9%) | S (100%)       |
| Phosphomycin | S (100%)| S (100%)       | S (100%)       |
| Genatmicin  | R (15.4%) S (15.4%) MS (69.2%) | R (18.2%) MS (81.8%) | S (25.0%) MS (75.0%) |
| Kanamyacin  | R (15.4%) S (84.6%) | R (9.1%) S (90.9%) | S (100%)       |
| Linkomycin  | MS (100%)| MS (100%)       | MS (100%)       |
| Oxitetracycline | S (100%)| S (100%)       | S (100%)       |
| Rifampicin  | MS (100%)| MS (100%)       | MS (100%)       |
| Spiramycin  | S (100%)| S (100%)       | S (100%)       |
| Streptomycin| S (100%)| S (100%)       | S (100%)       |
| Tetracycline| S (100%)| S (100%)       | S (100%)       |
| Tobramycin  | S (100%)| S (100%)       | S (100%)       |
| Vancomycin  | S (100%)| S (100%)       | S (100%)       |

R: Resistant to antibiotic; S: Sensitive to antibiotic; MS: Moderate sensitive to antibiotic.
of *Salmonella* spp. and *Salmonella Enteritidis*, but four of *Salmonella* Typhimurium were sensitive and the other four were resistant to cephalixin. Probably, this difference may be incurred because of the agents may have different genetics due to their polymorphic proteins, motile DNA particles such as transposons and plasmids, different intron-exon structures. The other probable cause of the occurrence of different resistance characteristics in the same strains may also be due to the ability of motile DNA particles to survive in extracellular region and some microorganisms, such as *Salmonella*, can integrate these particles into their genetic constitutions. 

In conclusion, meat meal, meat-bone meal, blood meal, chicken meal and feather meal samples produced under rendering procedures were analysed for *Salmonella* spp., *Salmonella Enteritidis* and *Salmonella Typhimurium*, and 13, 11 and 8 samples were positive respectively. While there were no any pathogens in the samples obtained from the place of production, some of the samples obtained from feed factories were positive. It is estimated that microbiological quality of rendered animal products are affected by processing technology and transportation from the place of production to the place of consumption.

REFERENCES

1. Pearl GG: The Future of Animal Proteins in Poultry Diets. Proceedings of the Multi-State Poultry Meeting, May 14-16, Atlanta, GA, USA, 2002.

2. European Community: Regulation (EC) No 1774/2002 of the European Parliament and of the Council of 3 October 2002 laying down health rules concerning animal by-products not intended for human consumption. Official Journal of the European Communities, 45, L 273: 1-95 EN 2002.

3. Official Gazette of Republic of Turkey: 28152, 24.01.2011 and 29585, 06.01.2016

4. EFSA: Microbiological risk assessment in feedingstuffs for food-producing animals. The EFSA Journal, 720, 1-84, 2008.

5. Akyar I, Can S: Comparison of “Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry” plus chromogenic media for detection of *Salmonella* spp. in gastrointestinal tract of animals. *Can J Microbiol*, 45, 965-970, 2009. DOI: 10.1077/kvf.2009.967

6. Milhosseini SZ, Seidavi A, Shivaazad M, Chamani M, Sadeghi AA, Pouresefy R: Detection of *Salmonella* spp. in gastrointestinal tract of broiler chickens by polymerase chain reaction. *Kafkas Univ Vet Fak Derg*, 15, 925-330, 2013. DOI: 10.9775/kvf.2012.7822

7. Jones FT: A review of practical *Salmonella* control measures in animal feed. *J Appl Poult Res*, 20, 102-113, 2011.

8. Franco DA: The Genus *Salmonella*. Proceedings of the Animal Protein Producers Industry. Institute for Continuing Education, 1-22, 1999.

9. Kinley B, Rieck J, Dawson P, Jiang X: Analysis of *Salmonella* and *enterococcus* isolated from rendered animal products. *Can J Microbiol*, 56, 65-73, 2010. DOI: 10.1139/W90-108

10. White PL, Naugle AL, Jackson CM, Fedorka-Cray PJ, Rose BE, Pritchard KM, Levine P, Saini PK, Schroder CM, Dreyfuss MS, Tan R, Holt KG, Harman J, Buchanan S: *Salmonella* Enteritidis in meat, poultry and pasteurized egg products regulated by the U.S. Food Safety and Inspection Service. 1998-2003. *J Food Prot*, 70, 582-91, 2007.

11. Mäde D, Petersen R, Trumper K, Stark R, Grohmann L: Inhouse validation of a real-time PCR method for rapid detection of *Salmonella* spp. in food products. *Eur Food Res Technol*, 219, 171-177, 2004. DOI: 10.1007/j.foodres.2010.08.012

12. Anonymous: Food and Drug administration. Bacteriological Analytical Manual. http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm070149.htm; Accessed: 28 November 2013.

13. Rahn K, De Grandis DS, Clarke RC, Mc Ewen SA, Galan JE, Cinocchio C, Curtiss R III, Gyles CL: 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, 1992. DOI: 10.1016/0890-8508(92)90002-f

14. Soumet C, Ermel G, Rose V, Rose N, Drouin P, Salvat G, Colin P: Evaluation of a multiplex-PCR-based assay for simultaneous identification of *Salmonella* sp., *Salmonella Enteritidis* and *Salmonella Typhimurium* from environment swabs of poultry houses. *Leit Appl Microbiol*, 29, 113-117, 1999. DOI: 10.1056/j.srep.2010.08.012

15. Doran JL, Collinson SK, Clouthier SC, Cebula TA, Koch WH, Burian J, Bansi PA, Todd ECD, Kay WW: Diagnostic potential of sefA DNA probes to *Salmonella* Enteritidis and certain other *Osegroup D1 Salmonella* serovars. *Mol Cell Probes*, 10, 233-246, 1996.

16. CLSI: Performance Standards for Antimicrobial Disk Susceptibility Test: Approved Standard-Eleventh Edition. Clinical and Laboratory Standards Institute Document M2A11[ISBN 1-56238-781-2], Clinical and Laboratory Standards Institute Institute 940 West Valley Road, Suite 1400, Wayne, PA 19087, USA, 2012.

17. Boyer CI, Brunner DW, Brown JA: *Salmonella* Organisms Isolated from Poultry Feed. *J Avian Dis*, 2, 396, 1958. DOI: 20.3829/1578479

18. CAST: Foodborne Pathogens: Risks and Consequences. Task Force Report No. 122. Ames, Iowa, Council of Agricultural Science and Technology, 1997.

19. Ockerman HW, Hansen CL: Animal by-product processing. Ellis Horwood Ltd. Chichester, England, 1988.

20. Trout H, Schaeffer D, Kakoma I, Pearl GG: Prevalence of Selected Foodborne Pathogens in Final Rendered Products. Fats and Proteins Research Foundation (FPRF), 312, Inc, Directors Digest, 2001.

21. Watkins JR, Flowers AI, Grumbles LC: *Salmonella* organisms in animal products used in poultry feeding. *Avian Dis*, 3, 290, 1959. DOI: 20.3829/1578476

22. Pomeroy BS, Grady MK: *Salmonella* Organisms Isolated from Feed Ingredients. Proc US Livestock Sanit Ass, 65, 449, 1961.

23. Ge B, LaFon PC, Carter PJ, McDermott SD, Abbott J, Glenn A, Ayers SL, Friedman SL, Paige JC, Wagner DD, Zhao S, McDermott PF, Rasmussen MA: Retrospective analysis of *Salmonella*, *Campylobacter*, *Escherichia coli*, and *Enterococcus* in animal feed ingredients. *Foodborne Pathog Dis*, 10, 684-691, 2013. DOI: 10.1089/fpd.2012.1470

24. Stojanac N, Stancic A: Estimation of the *Salmonella* spp. prevalence in pig farms with dry and wet feeding. *Afr J Microbiol Res*, 7, 3272-3274, 2013.

25. Laban SE, Moustafa GZ, Anwer W, Badawy EM: Microbial load of poultry by-products following rendering process. *Global Veterinaria*, 12, 756-759, 2014.

26. Sutton AL, Scheidt AB, Patterson JA: Final Research Report. Fats and Protein Research Foundation, 1992.

27. Capita R, Alonso-Calleja C: Antibiotic-resistant bacteria: a challenge for the major food industry. *Crit Rev Food Sci Nutr*, 53, 11-48, 2013. DOI: 10.1080/10408398.2010.519837

28. Richens J: Major tropical syndromes by body system: The gastrointestinal tract. Typhoid fever. In, Armstrong D, Cohen J (eds). Infectious diseases. 241, Mosby, London, 1999.

29. Rowe B, Ward LR, Threlfall EJ: Multidrug resistant *Salmonella typhi*: A worldwide epidemic. *Clin Infect Dis*, 24, 106-109, 1997. DOI: 10.1089/clinics/24.supplement.1.106

30. Kohinur B, Reza AT, Haque Hossain A, Hassan NM, Akhter N, Ahmed A, Barua U: Isolation, identification and antibiotic resistance pattern of *Salmonella* spp. from chicken eggs, intestines and environmental samples. *Bangladesh Pharm J*, 13, 23-27, 2010.

31. Yildirim Y, Gonulalan Z, Pamuk S, Ertas N: Incidence and antibiotic
32. Zarakolu P, Karabicak N, Oncul O, Guvener E: In vitro antimicrobial resistance of Salmonella Typhimurium isolates. Bull Microbiol, 30, 125-128, 1996.

33. Dallal MMS, Doyle MP, Rezadehbashi M, Dabiri H, Sanaei M, Modaressi S, Bakhtiari R, Sharify K, Taremi M, Zali MR, Sharifi-Yazdi MK: Prevalence and antimicrobial resistance profiles of Salmonella serotypes, Campylobacter and Yersinia spp. isolated from retail chicken and beef, Tehran, Iran. Food Control, 21, 388-392, 2010.

34. Yan H, Li L, Alam MJ, Shinoda S, Miyoshi S, Shi L: Prevalence and antimicrobial resistance of Salmonella in retail foods in northern China. Int J Food Microbiol, 143, 230-234, 2010. DOI: 10.1016/j.ijfoodmicro.2010.07.034

35. Del Rio E, Panizo-Morán M, Prieto M, Alonso-Calleja C, Capita R: Effect of various chemical decontamination treatments on natural microflora and sensory characteristics of poultry. Int J Food Microbiol, 115, 268-280, 2007.