

# Prevalence and Antimicrobial Susceptibility of *Salmonella* in Rendered Animal Products Used in Poultry Feed in Turkey <sup>[1]</sup>

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## 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, flumequin, fosfomycin, kanamycin, oxytetracycline, spiramycin, streptomycin, tetracycline, tobramycin and vancomycin and moderate sensitive to gentamicin, linkomycin and rifampicin.

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

## Türkiye’de Kanatlı Yemlerinde Kullanılan Rendering Ürünlerde *Salmonella* Varlığı ve Bunların Antimikrobiyal Duyarlılığı

### Özet

Kanatlılarda artan *Salmonella* bulaşıklığı son on yıldır bilimsel yönden büyük önem kazanmıştır. Bu çalışmada Türkiye’deki çeşitli yem fabrikaları ve üretim noktalarından toplam 500 adet hayvansal rendering ürünü (et unu, et-kemik unu, kan unu, tavuk unu ve tüy unu) toplanmış ve bu örnekler *Salmonella* spp., *Salmonella Enteritidis* and *Salmonella Typhimurium* bakımından analiz edilmiştir. Sonuçlara göre yem fabrikalarından elde edilen 13, 11 ve 8 örnek sırasıyla *Salmonella* spp., *Salmonella Enteritidis* and *Salmonella Typhimurium* bakımından pozitif olarak tespit edilmiştir. Bununla birlikte üretim noktalarından toplanan tüm örnekler ise negatif sonuç vermiştir. Pozitif olan izolatların antibiyotik duyarlılıkları 17 farklı antibiyotik kullanılarak tayin edilmiştir. *Salmonella* spp. ve *Salmonella Enteritidis* serovarlarının amikasin, sefazolin ve eritromisin’e dirençli, amoksisilin, kloramfenikol, flumequin, fosfomisin, kanamisin, oksitetrasiklin, spiramisin, streptomisin, tetrasiklin, tobramisin ve vankomisin’e duyarlı, gentamisin, linkomisin ve rifampisin’e orta derecede duyarlı olduğu tespit edilmiştir.

**Anahtar sözcükler:** *Salmonella*, Kanatlı, Rendering ürünleri, Antibiyotik, Duyarlılık

## 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 <sup>[1]</sup>. In Turkey,

rendering by-products have been used intensively in poultry diets because of their quality protein, calcium and utilizable 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



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livestock feeding was banned in 2002 by the European Union [2]. In Turkey, the use of rendered animal products in poultry nutrition were banned by Republic of Turkey Ministry of Food, Agriculture and Livestock in 01/01/2016. However, this prohibition were delayed until 01/01/2017 [3].

Inadequate hygienic conditions in the production and storage of feedstuffs and primer and secondary factors during the slaughter process of animals may lead to microbiological contamination of the obtained products. The most important subject in the use of rendered animal products is the prevention of microbiological contamination. Beside oil seed meals, European Food Safety Authority (EFSA) reported that animal derived proteins were the major risk feed materials for introducing *Salmonella* contamination to feed mills and industrial compound feed [4]. *Salmonella* species are gram-negative facultative intracellular bacteria and they have a wide spectrum of diseases [5]. Salmonellosis is one of the most frequent foodborne diseases, being an important public health problem in almost all industrialized countries [6].

Although the risk of *Salmonella* spp. in rendered animal products always available, the temperatures used during the feed production are normally sufficient to eliminate *Salmonella* spp. [7]. In spite of the fact that *Salmonella* contamination is the potential risk for all feedstuffs, only a few of serotype of 2300 identified *Salmonella* are related to clinical symptoms in animals and humans. Also, *Salmonella* strains are not resistant to physical and chemical agents and they deactivate in 1 h at 55°C or in 15-20 min at 60°C [8].

However, the secondary contaminations during the transportation from rendering plant to feed factories and storage are the major problem in *Salmonellosis*. In a study, it was reported that there were any *Salmonellosis* agent in rendering samples obtained from production point, but 8.7% of transported and stored samples were positive for *Salmonella* spp. [9].

Due to the increase in antibiotic-resistant pathogens, the use of antibiotics in poultry feeds were banned legally in Europe Union and in the present country also accepted this decision by considering human and animal health. However, expanding in the global market for livestock, feed, feed additive and food can still lead to the spread of several *Salmonella* serotypes and lead to the increase in the incidence of *Salmonella* spp. infections [10].

The aim of this study was to investigate the existence of *Salmonella* spp., which is considered as an important risk for poultry and consumer health in the worldwide and also Turkey, in poultry feeds contained potential infected rendered animal products, to determine the types of identified strains serologically by specifying the incidence of *S. Typhimurium* and *S. Enteritidis*, which are the most dangerous species, and to determine the antibiotic susceptibility of these species.

## 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 parallelly 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 [11].

### 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" antisera, 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 antisera [12].

### PCR Analysis

The primer sequences used in PCR analysis for *Salmonella* spp. [13], *Salmonella* Typhimurium [14] and *Salmonella* Enteritidis [15] are shown in Table 1. PCR mix was as follows (final 25 µL); 2 µL DNA samples, 2.5 mM MgCl<sub>2</sub>, 10 mM Tris-HCl pH 8.0, 5 mM KCl (0.2 mM from each nucleotide), each

**Table 1.** The properties of primer sequences designed according to different *Salmonella* serotypes**Tablo 1.** Farklı serotiplere göre dizayn edilen primer dizileri ve özellikleri

| Primers (5' – 3')           | Properties  |             |          | Target Microorganism          |
|-----------------------------|-------------|-------------|----------|-------------------------------|
|                             | Gene        | Length (bp) | Amp (bp) |                               |
| GTGAAATTATCGCCACGTTCCGGGCAA | <i>invA</i> | 26          | 284      | <i>Salmonella</i> spp.        |
| TCATCGCACCGTCAAAGGAACC      | <i>invA</i> | 22          | 284      |                               |
| CGGTGTTGCCAGGTTGGTAAT       | <i>fliC</i> | 22          | 620      | <i>Salmonella</i> Typhimurium |
| ACTGGTAAAGATGGCT            | <i>fliC</i> | 16          | 620      |                               |
| AGCCAACCATTGCTAAATTGG       | <i>invA</i> | 21          | 488      | <i>Salmonella</i> Enteritidis |
| CGGTAATCAGCATCTGCAGTAGC     | <i>sefA</i> | 24          | 488      |                               |

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. Amplication 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; flumoquin, 30 µg; phospho-mycin, 50 µg; kanamycin, 30 µg; oxytetracycline, 30 µg; spiramycin, 100 µg; streptomycin, 10 µg; tetracycline, 30 µg; tobramycine, 30 µg; vancomycin, 30 µg; gentamicin, 10 µg; linkomycin, 10 µg; rifampicin, 5 µg; amikacin, 30 µg; cephalosporin, 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 analysed 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, cephalosporin and erythromycin, sensitive to amoxicillin, chloramphenicol, flumoquin, 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 pathogens such as *Salmonella* strains, *Listeria monocytogenes*, *Campylobacter jejuni* and *Clostridium perfringens* contamination were detected in raw materials 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 pathogens 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

**Tablo 2.** Çeşitli yem fabrikaları ve üretim noktalarından alınan rendering örneklerinde *Salmonella* spp., *Salmonella* Enteritidis and *Salmonella* Typhimurium kontaminasyonu

| Samples Obtained from Feed Factories          | Occurrence | Incident Rate, % | Isolation and Identification (+)/(-) | PCR Verification (+)/(-) |
|---|------------|------------------|--------------------------------------|--------------------------|
| <b><i>Salmonella</i> spp.</b>                 |            |                  |                                      |                          |
| 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                   |
| <b><i>Salmonella</i> Enteritidis</b>          |            |                  |                                      |                          |
| 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                   |
| <b><i>Salmonella</i> Typhimurium</b>          |            |                  |                                      |                          |
| 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                    |
| <b>Samples Obtained from Rendering Plants</b> |            |                  |                                      |                          |
| <b><i>Salmonella</i> spp.</b>                 |            |                  |                                      |                          |
| Meat meal                                     | 0/20       | 0                | 0/20                                 | 0/20                     |
| Meat-bone meal                                | 0/20       | 0                | 0/20                                 | 0/20                     |
| Blood meal                                    | 0/20       | 0                | 0/20                                 | 0/20                     |
| Chicken meal                                  | 0/20       | 0                | 0/20                                 | 0/20                     |
| Feather meal                                  | 0/20       | 0                | 0/20                                 | 0/20                     |
| Total   | 0/100      | 0                | 0/100                                | 0/100                    |
| <b><i>Salmonella</i> Enteritidis</b>          |            |                  |                                      |                          |
| Meat meal                                     | 0/20       | 0                | 0/20                                 | 0/20                     |
| Meat-bone meal                                | 0/20       | 0                | 0/20                                 | 0/20                     |
| Blood meal                                    | 0/20       | 0                | 0/20                                 | 0/20                     |
| Chicken meal                                  | 0/20       | 0                | 0/20                                 | 0/20                     |
| Feather meal                                  | 0/20       | 0                | 0/20                                 | 0/20                     |
| Total   | 0/100      | 0                | 0/100                                | 0/100                    |
| <b><i>Salmonella</i> Typhimurium</b>          |            |                  |                                      |                          |
| Meat meal                                     | 0/20       | 0                | 0/20                                 | 0/20                     |
| Meat-bone meal                                | 0/20       | 0                | 0/20                                 | 0/20                     |
| Blood meal                                    | 0/20       | 0                | 0/20                                 | 0/20                     |
| Chicken meal                                  | 0/20       | 0                | 0/20                                 | 0/20                     |
| Feather meal                                  | 0/20       | 0                | 0/20                                 | 0/20                     |
| Total   | 0/100      | 0                | 0/100                                | 0/100                    |

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

| Salmonella spp. Positive |              | Salmonella Enteritidis Positive |              | Salmonella Typhimurium Positive |              |
|--------------------------|--------------|---------------------------------|--------------|---------------------------------|--------------|
| 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                                    | Flum                   | Phos | Gent                           | Kana                 | Link | Oxit | Rifa | Spir | Stre | Tetr | Tobr | Vanc |
|----------------|----------------|------|------------------------|------------------------------|---|------------------------|------|--------------------------------|----------------------|------|------|------|------|------|------|------|------|
| R              | 1-13<br>17-30  | -    | 1-15<br>18-24<br>29-32 | 11<br>20-21<br>31            | 1-6<br>10-18<br>20-21<br>23-27<br>29-31 | 11-12<br>17            | -    | 7-8<br>17-18                   | 7-8<br>19            | -    | -    | -    | -    | -    | -    | -    | -    |
| S              | 14-16<br>31-32 | 1-32 | 16-17<br>25-28         | 1-10<br>12-19<br>22-30<br>32 | 7-9<br>19<br>22<br>28<br>32             | 1-10<br>13-16<br>18-32 | 1-32 | 9-10<br>25-26                  | 1-6<br>9-18<br>20-32 | -    | 1-32 | -    | 1-32 | 1-32 | 1-32 | 1-32 | 1-32 |
| MS             | -              | -    | -                      | -                            | -                                       | -                      | -    | 1-6<br>11-16<br>19-24<br>27-32 | -                    | 1-32 | -    | 1-32 | -    | -    | -    | -    | -    |

R: Resistant to antibiotic; S: Sensitive to antibiotic; MS: Moderate sensitive to antibiotic; Amik: Amikacin; Amox: Amoxicillin; Ceph: Cephalosporin; Chlo: Chloramphenicol; Eryt: Erythromycin; Flum: Flumequin; Phos: Phosphomycin; Gent: Gentamicin; Kana: Kanamycin; Link: Linkomycin; Oxit: Oxitetracycline; Rifa: Rifampicin; Spir: Spiramycin; Stre: Streptomycin; Tetr: Tetracycline; Tobr: Tobramycin; Vanc: Vancomycin

appropriate time-temperature can inactivate large group of food pathogens 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 5.** Antibiotic susceptibility percentages of *Salmonella* strains isolated from 32 positive rendering samples**Tablo 5.** 32 pozitif renderin örneğinden izole edilen *Salmonella* türlerinin antibiyotiğe duyarlılık yüzdeleri

| Antibiotics     | <i>S. spp.</i>                 | <i>S. Enteritidis</i> | <i>S. Typhimurium</i> |
|-----------------|--------------------------------|-----------------------|-----------------------|
| Amikacin        | R (100%)                       | R (72.7%) S (27.3%)   | R (75.0%) S (25.0%)   |
| Amoxicillin     | S (100%)                       | S (100%)              | S (100%)              |
| Cephazolin      | 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%)   |
| Fluoroquinolone | R (15.4%) S (84.6%)            | R (9.1%) S (90.9%)    | S (100%)              |
| Phosphomycin    | S (100%)                       | S (100%)              | S (100%)              |
| Gentamicin      | R (15.4%) S (15.4%) MS (69.2%) | R (18.2%) MS (81.8%)  | S (25.0%) MS (75.0%)  |
| Kanamycin       | R (15.4%) S (84.6%)            | R (9.1%) S (90.9%)    | S (100%)              |
| Linkomycin      | MS (100%)                      | MS (100%)             | MS (100%)             |
| Oxytetracycline | 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*

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 ofloxacin. 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%), sulfamethoxazole/trimethoprim (48.1%), nalidixic acid (30.9%), tetracycline (19.8%), carboxybenzylpenicillin (17.3%), amoxicillin (17.3%) and ampicillin (16.0%) and also 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, cephazolin and erythromycin. However, isolated *Salmonella* spp. and *Salmonella* Enteritidis serovars were resistant to amikacin, cephazolin and erythromycin, sensitive to amoxicillin, chloramphenicol, fluoroquinolone, 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 cephazolin, were similar to those

of *Salmonella* spp. and *Salmonella* Enteritidis, but four of *Salmonella* Typhimurium were sensitive and the other four were resistant to cephazolin. 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<sup>[15,35]</sup>.

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.

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