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# Presence of *Staphylococcus aureus* and Shiga Toxigenic *Escherichia coli* O157:H7 in Raw Meat in Ağrı, Turkey

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**Abstract**

**Background:** *Staphylococcus aureus* and Shiga toxin producing *Escherichia coli* O157:H7 (EHEC) are significant foodborne pathogens worldwide. While *S. aureus* can cause mild superficial skin infections or life-threatening bacteremia and endocarditis, as well as toxin-induced cases such as toxic shock syndrome; *E. coli* O157:H7 can cause symptoms from mild diarrhea to severe hemorrhagic colitis (HC), hemolytic uremic syndrome (HUS), thrombotic thrombocytopenic purpura (TTP).

**Objectives:** The objectives of this study were to find out the prevalence and seasonal distribution of *S. aureus* in 214 frozen raw meat (turkey, chicken and beef) and the prevalence of *E. coli* O157:H7 in 70 raw beef with the characterization of the *E. coli* O157:H7 isolate by multiplex polymerase chain reaction (PCR).

**Materials and Methods:** For the detection of *S. aureus*, a total of 214 frozen raw meat samples including 74 turkey meat, 70 chicken meat and 70 beef cuts (approximately 2 × 3 cm cubic parts); and for the detection of *E. coli* O157:H7, a total of 70 frozen raw beef samples that all were produced from national companies and consumed in Ağrı, Turkey were analyzed.

**Results:** Out of 214 meat samples, 25.7 % (18/70) of the beef, 11.4 % (8/74) of the chicken meat, and 5.4 % (4/70) of the turkey meat samples were contaminated with *S. aureus*. Out of 70 frozen raw beef samples, only 1 (1.4%) was identified as both Shiga toxin 1 and 2 producing *E. coli* O157:H7 by the detection of *stx1*, *stx2*, *eaeA*, *hly*, and $fliC_{h7}$ according to multiplex PCR analysis.

**Conclusion:** Our findings demonstrate that occurrence frequency of *S. aureus* was higher in frozen raw beef than in raw chicken and turkey meat samples. Although the prevalence of *E. coli* O157:H7 was low in beef, the presence of virulence genes, especially toxin genes remain a significant public health concern.

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## Background

*Staphylococcus aureus* is one of the most significant food pathogen responsible for intoxications worldwide.[1] Clinically, *S. aureus* can cause mild superficial skin infections or life-threatening bacteremia and endocarditis, as well as toxin-induced cases such as toxic shock syndrome.[2] *S. aureus* produces more than 30 different extracellular components and some *S. aureus* strains are able to produce staphylococcal enterotoxins (SEs) in foods and can be the causative agents of staphylococcal food poisonings.[1] The prevalence of *S. aureus* may range from 1% to 80% in various foods. In addition, it was reported that turkey meat and beef were the most prevalent foods for the incidence of *S. aureus*.[3]

Intestines are an important vehicle of bacteria and as a result during slaughtering and processing, raw meats are often contaminate with feces of animals.[4] At every stage, from fork to table, contamination of food with enterotoxigenic staphylococci can take place due to poor personal hygiene which causes colonized people (30%-50%) to supply the main source of dissemination of staphylococci.[5] Mead et al[6] reported that, foodborne pathogens cause 76 million of illnesses cases, 325 000 hospitalizations, and 5000 deaths in the United States annually. Among these, 0.1% of food-related deaths are caused by *S. aureus*.[6] Contaminated raw or undercooked poultry and red meat are exclusively important in transmission of these foodborne pathogens.[7]

Shiga toxin producing *E. coli* that classified as enterohemorrhagic *Escherichia coli* O157:H7 (EHEC) is associated with food and water borne infections. Recent studies had implicated *E. coli* O157:H7 as a foodborne pathogen

throughout the world and in recent years especially in Europe, the Unites States, South Africa, and Japan *E. coli* O157:H7 has been accepted as one of the most important foodborne pathogen concerning public health.[8] The primary reservoir is considered to be cattle.[9] Generally, cattle are shedding *E. coli* O157:H7 in their feces without showing any symptoms.[10] In many studies on the microbiological hygiene of cattle at slaughter have shown that, hides are the main vehicles for contamination of the pathogens to carcasses.[11-13] Omisakin et al[14] reported that, undercooked beef and beef products have often played significant role in foodborne *E. coli* O157:H7 infections. This makes *E. coli* O157:H7 an important foodborne pathogen, because cattle are the main source of red meat (432.406 tons) in Turkey with a rate of 88% of total red meat production (1.008.272 tons).[15] In numerous studies worldwide, prevalence of *E. coli* O157:H7 in raw meats and meat products was reported as 0.12%,[16] 1.1%,[17,18] 2.3%,[19] and 2.8%.[20]

*Escherichia coli* O157:H7 can cause symptoms from mild diarrhea to severe hemorrhagic colitis (HC), hemolytic uremic syndrome (HUS), thrombotic thrombocytopenic purpura (TTP).[18] HUS causes acute renal failure in children worldwide. The main virulence factors of *E. coli* O157:H7 are Sshiga toxins 1 and 2 encoded by *stx1* and *stx2* genes, respectively. *E. coli* O157:H7 can cause HUS mainly by secretion of these Shiga toxins. In addition to its toxins, the *eaeA* gene which encodes intimin, a 94–97 kDa outer membrane protein, is responsible for adherence to the intestinal mucosa.[16,21,22] Also, enterohemolysin encoded by *hlyA*[21] and flagellar H7 encoded by *fliC*$_{h7}$[23] are the other virulence factors that are responsible in pathogenicity of *E. coli* O157:H7.

## Objectives

The objectives of this study were to investigate the prevalence of *S. aureus* in frozen raw chicken meat, turkey meat, and beef samples that were produced from national companies, and also the presence of *E. coli* O157:H7 in frozen raw beef in Ağrı, Turkey with determining the main virulence determinants of *E. coli* O157:H7 isolated from a beef sample by multiplex PCR.

## Materials and Methods

### Sampling

For the detection of *S. aureus,* a total of 214 frozen raw meat samples including 70 chicken meat, 74 turkey meat, and 70 beef cuts (app. 3 × 2 cm cubic parts); and for the detection of *E. coli* O157:H7, a total of 70 frozen raw beef samples that all were produced from national companies and consumed in Ağrı, Turkey were analyzed. Samples were immediately transported to the laboratory in an ice bag and analyzed within the same day.

### Isolation and Identification of *Staphylococcus aureus*

For the isolation of *S. aureus*, 10 g of meat samples were weighed into a sterile bag containing 90 mL of buffered peptone water (BPW; Oxoid CM1049, Basingstoke, UK) and homogenized. Afterwards, aliquots of 1 mL were plated on Baird Parker agar (BP; Oxoid CM0275) supplemented with egg yolk tellurite emulsion (Oxoid SR0054), and incubated at 37°C for 24-48 hours. After incubation, up to five typical colonies (circular, smooth, convex, moist, 2-3 mm in diameter, gray to jet-black, frequently with light-colored margin, surrounded by opaque zone and frequently with a clear outer zone) were picked and resuspended in 5 mL of Brain Heart Infusion Broth (BHI, Oxoid CM0225), and incubated at 35°C for 18-24 hours. At the end of the incubation, 0.2 mL of cell suspensions were transferred to a tube containing 0.6 mL of coagulase plasma (rabbit) with EDTA (Bactident Coagulase, Merck 1.13306, Darmstadt, Germany), mixed thoroughly, incubated at 35°C, and examined periodically over a 6-hour period for clot formation. Only firm and complete clot that stays in place when tube was tilted or inverted was considered as coagulase positive *Staphylococcus*. Coagulase positive isolates were tested with Dryspot Staphytect Plus kit for the identification of *S. aureus* (Oxoid DR0100M). According to the Dryspot test, agglutination reaction positive isolates were identified as *S. aureus*.[24] In the study, for the isolation and identification of *S. aureus*, reference strain *S. aureus* ATCC 25923 was used as positive control.

### Isolation and Identification of *Escherichia coli* O157

Isolation procedure was performed according to ISO 16654.[25] Twenty-five grams of beef samples were weighted to sterile bags and enriched with 225 mL EC broth (Oxoid CM0853B, Hampshire, UK) supplemented with novobiocin (Oxoid SR0181E) and incubated at 37 °C for 18 hours. Then, 0.1 mL of enrichment was plated on Sorbitol MacConkey agar (Oxoid CM0813B) plates supplemented with Cefixime-tellurite (Oxoid SR0172E). After 24 hours incubation at 37°C, sorbitol negative colonies were tested for the O157 antigen by latex agglutination (Oxoid DR0620). Agglutination positive colony was approved as *E. coli* O157 and then taken for multiplex PCR analysis for the detection of H7 (*fliC*$_{h7}$) and virulence genes (*stx1, stx2, eaeA* and *hly*).

### Multiplex Polymerase Chain Reaction Analysis of *Escherichia coli* O157 Isolate

In the study, the presences of *H7* (*fliC*$_{h7}$) and virulence genes (*stx1, stx2*, *eaeA* and *hly*) of *E. coli* O157 isolate were analyzed by multiplex PCR assay. DNA extraction was performed using Chelex-100 (Bio-Rad, Hercules, CA, USA) resin based technique as described in our previous study.[26] Multiplex PCR assay was performed according to Fratamico et al[27] by 5 different primer pairs (SLT1 - F, SLT1 - R; SLTII - F, SLTII - R; AE22, AE20 - 2; MFS1 - F, MFS1 - R and FLICH7 - F, FLICH7 - R) (Thermo Electron, MA, USA) to identify *E. coli* O157:H7 and to determine the virulence factors of the isolate. *E. coli* O157:H7 ATCC 43895 reference strain that is positive for *stx1, stx2, eaeA, hly* and *fliC*$_{h7}$ genes was used as positive control.

For the amplification, PCR master mix was prepared with a final volume of 50 µL containing incomplete 1 ×

Reaction Buffer (Bioron GmbH, Ludwigshafen, Germany), 3.0 mM $MgCl_2$ (Bioron), 400 µM each of the four deoxynucleoside triphosphates (dNTPs) (Bioron), 2.5 U *Taq* DNA polymerase (Bioron), 0.50 µM of all primers except AE22 and AE20 - 2 that were used 0.25 µM. Then 10 µL of DNA was added to reaction mixture. Thermal cycling (Eppendorf Mastercycler Gradient, Hamburg, Germany) was carried out with the initial denaturation at 94°C for 2 minutes and then 35 cycles of denaturation at 94°C for 20 seconds, annealing at 54°C for 1 minute, and extension at 72°C for 1 minute, with a final extension for 10 minutes at 72°C.[27] Following to the thermal cycling, 10 µL of PCR products stained with 2 µL of 6 × loading dye (Promega, Madison, USA) was subjected to 1.5% agarose gel (Agarose-Basica LE, Prona, Spain) containing 0.1 µg/mL ethidium bromide (BioChemica GmbH, Darmstadt, Germany) and electrophoresis (CSL MSMixi - Duo, Corston, UK) was performed for 1 hour at 100 V. Amplicon visualization and documentation was performed using gel documentation system (Syngene Ingenius, Cambridge, UK).

## Results

### Results for Staphylococcus aureus

In the study, a total of 214 frozen raw meat samples including 74 turkey meats, 70 chicken meats and 70 beef were tested for the presence of *S. aureus*. Out of 214 meat samples 30 (14.0%) harbored *S. aureus*. Out of 70 beef, 70 chicken meat, and 74 turkey meat samples, 18 (25.7%), 8 (11.4%), and 4 (5.4%) were contaminated with *S. aureus*, respectively.

As far as seasonal distribution was concerned, during the spring four (18.2%) of the 22 beef, 2 (10.5%) of the 19 chicken meat, and 2 (13.3%) of the 15 turkey meat samples were contaminated with *S. aureus*. In the summer, 6 (35.3%) of the 17 beef and 1 (6.3%) of the 16 chicken meat samples were contaminated with *S. aureus*. None of the 24 turkey meat samples analyzed in the summer harbored *S. aureus*. During the autumn, from seven (50.0%) of the 14 beef, 2 (11.8%) of the 17 chicken meat, and 1 (5.3%) of the 19 turkey meat samples *S. aureus* was detected while, during the winter, from 1 (5.9%) of the 17 beef, 3 (16.7%) of the 18 chicken meat, and 1 (6.3%) of the 16 turkey meat samples *S. aureus* was isolated. In general, 14.3% (8/56), 14.0% (8/57), 20.0% (10/50), and 9.8% (5/51) of the meat samples were found to be contaminated with *S. aureus* during the spring, summer, autumn, and winter, respectively (Table 1). The lowest prevalence rates were detected in winter months.

Table 1. Seasonal Distribution of *Staphylococcus aureus* in Frozen Raw Meats in Ağrı

### Results for *Escherichia coli* O157

A total of 70 frozen raw beef samples were analyzed for the presence of *E. coli* O157 and only from 1 (1.4%) of the sample which was collected in the spring, *E. coli* O157 was isolated.

Multiplex PCR assay was performed for the detection of H7 (*fliC*$_{h7}$) and virulence genes (*stx1, stx2, eaeA* and *hly*) from the isolate. Finally both five virulence genes were detected (Figure 1). The isolate was identified as both Shiga toxin 1 and 2 producing *E. coli* O157:H7 by the detection of *stx1*, *stx2*, *eaeA*, *hly*, and *fliC*$_{h7}$ (Table 2).

## Discussion

It was found that, 25.7% (18/70) of the beef, 11.4% (8/74) of the chicken meat, and 5.4% (4/70) of the turkey meat samples analyzed were contaminated with *S. aureus*. These results showed that, beef had the highest *S. aureus* prevalence with regards to the chicken and turkey meat samples. In previous studies the prevalence of *S. aureus* in beef and poultry meat was ranges from 20.0% to 25.0% and 11.4% to 65.8% in beef and chicken meats,

**Table 1.** Seasonal Distribution of *Staphylococcus aureus* in Frozen Raw Meats in Ağrı

| Season/Date | Number of Samples | | | | | |
|---|---|---|---|---|---|---|
| | Chicken Meat | | Turkey Meat | | Beef | |
| | Analyzed | *S. aureus* Positive | Analyzed | *S. aureus* Positive | Analyzed | *S. aureus* Positive |
| Summer | | | | | | |
| June | 5 | - | 9 | - | 6 | 2 |
| July | 5 | 1 | 8 | - | 4 | 2 |
| August | 6 | - | 7 | - | 7 | 2 |
| Autumn | | | | | | |
| September | 5 | 1 | 7 | 1 | 5 | 4 |
| October | 6 | - | 8 | - | 5 | 2 |
| November | 6 | 1 | 4 | - | 4 | 1 |
| Winter | | | | | | |
| December | 7 | 1 | 5 | - | 6 | - |
| January | 6 | 2 | 5 | 1 | 5 | 1 |
| February | 5 | - | 6 | - | 6 | - |
| Spring | | | | | | |
| March | 7 | 1 | 4 | 1 | 9 | 1 |
| April | 5 | - | 5 | - | 7 | 1 |
| May | 7 | 1 | 6 | 1 | 6 | 2 |
| Total | 70 | 8 | 74 | 4 | 70 | 18 |

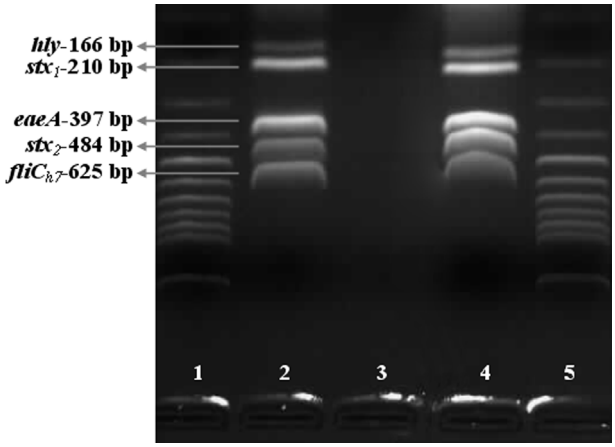


**Figure 1.** Virulence Gene Profile of *E. coli* O157:H7 Isolate by Multiplex PCR.
Lanes 1 and 5: 100 bp DNA marker; Lane 2: positive control, *E. coli* O157:H7 ATCC 43895; Lane 3: negative control. Lane 4: *stx1*, *stx2*, *eaeA*, *hly,* and $fliC_{h7}$ genes positive isolate.

respectively.[28,29]

In Australia, after evisceration of beef carcasses during slaughtering, from 40% of the samples coagulase positive *Staphylococcus* was detected.[30] In Japan, 77.8% of beef and 91.7% of chicken meat samples collected from markets were contaminated with *S. aureus*.[31] Prevalence of *S. aureus* in raw meat species is relatively higher in China and Japan than Turkey.

In our previous study of *E. coli* O157:H7 similar findings were detected, it was found that 2 (0.79%) of the 251 fresh ground beef samples were contaminated with *E. coli* O157:H7.[26] Furthermore in another study from Turkey, *E. coli* O157:H7 was not detected in beef products.[32] Similar to the present study, the prevalence of *E. coli* O157:H7 in ground beef was reported around 1.0%-1.1% in various countries including the Netherlands,[33] Spain,[34] and the United Kingdom,[35] contrarily higher prevalence rates ranging from 2.8% to 16.8% in beef samples were reported in Argentina,[36] Ireland,[20] and the United States.[37] However, from the United States, higher prevalence (14.2%) for *E. coli* O157:H7 in cattle was reported.[38] Also in a study conducted in Iran, *stx* gene harboring *E. coli* strains were detected from 5%, out of 125 frozen food samples of animal origin (chicken, fish, mince, slice kebab meat, and beef burger).[39] Although most *E. coli* O157:H7 infections occur in the summer and autumn,[40] in the present study *E. coli* O157:H7 was isolated from frozen raw beef samples in the spring. Also a similar finding to the present study about the seasonal variation of *E. coli* O157:H7 in cattle was reported. In England higher *E. coli* O157:H7 prevalence was detected in the spring (38%) than during the winter months (4.8%) from cattle.[12]

Molecular characterization of the *E. coli* O157:H7 isolate was performed by multiplex PCR and five virulence genes including *stx1, stx2, eaeA, hly,* and $fliC_{h7}$ were detected. In our previous study, from one of the two isolates, all *stx1, stx2, eaeA, hly,* and $fliC_{h7}$ genes were found, while the other isolate harbored only *stx1, eaeA, hly,* and $fliC_{h7}$

**Table 2.** Prevalence and Virulence Genes Profile of *E. coli* O157:H7 in Frozen Raw Beef in Ağrı

| Season/Sample Collection Dates | No. of Analyzed Samples | *E. coli* O157:H7 Positive Sample | Virulence Gene Profile of the Isolate |
|---|---|---|---|
| Summer | | | |
| June | 6 | - | - |
| July | 4 | - | - |
| August | 7 | - | - |
| Autumn | | | |
| September | 5 | - | - |
| October | 5 | - | - |
| November | 4 | - | - |
| Winter | | | |
| December | 6 | - | - |
| January | 5 | - | - |
| February | 6 | - | - |
| Spring | | | |
| March | 9 | 1 | *stx1, stx2, eaeA, hly,* $fliC_{h7}$ |
| April | 7 | - | - |
| May | 6 | - | - |
| Total | 70 | 1 | |

genes.[26] In a different study, 43 of the 1533 beef samples were determined as *E. coli* O157:H7 and from all of the isolates, *eaeA*, *hly* and $fliC_{h7}$ genes and from only from 41 of the isolates both *stx1* and *stx2* genes were determined.[20] Vernozy-Rozand et al[41] detected *E. coli* O157:H7 from 4 of the 3450 ground beef samples and from all of the isolates *stx1*, *stx2*, *eaeA* and *ehx* genes were detected. In a study performed in Ireland, 29 of the 30 *E. coli* O157:H7 isolated from beef trimmings have *eaeA* and *hlyA* genes while in 13/30 *E. coli* O157:H7 only *stx1*, in 14/30 only *stx2* and in 2/30 isolates both *stx1* and *stx2* genes were detected. Surprisingly, in one *E. coli* O157:H7 none of the virulence genes including *stx1*, *stx2*, *eaeA* and *hlyA* were found.[42] This finding is not in agreement with our results. It was reported that this differences may caused by the ability of losing genes, including Shiga toxin genes, of *E. coli* O157:H7 during subcultivation and such differences may cause changes in virulence of pathogen during storage and processing.[43] In a study that Badouei et al[44] investigated the presence of major virulence genes of EHEC (*stx1, stx2, eae, Ehly*) from 158 diarrhoeic calves in Iran, 13 (8.2%) calves carried strains positive for one or more of the virulence factors tested, and 11 (6.9%) calves were contaminated with *stx1* or *stx2* harbored strains. However none of the EHEC were identified as O157:H7.[44] In another study, 18 non-sorbitol fermenting *E. coli* isolated from 180 clinically healthy cattle fecal samples. Although two isolates harbored the tested virulence genes (one *stx2* and *Ehly*, and the other one *stx2, eae* and *Ehly*), none of them belonged to O157 serogroup.[45]

According to the analysis results, occurrence frequency

of *S. aureus* was detected higher in frozen raw beef than in raw chicken and turkey meat samples. The presence of *S. aureus* in raw meat is a potential risk for public health. *E. coli* O157:H7 was detected only in one of the 70 frozen beef samples and major virulence genes *stx1*, *stx2*, *eaeA*, *hly*, and $fliC_{h7}$ were determined from the isolate. Although the prevalence of *E. coli* O157:H7 was low in beef in Ağrı, the presence of virulence genes, especially toxin genes remain a significant public health concern. Therefore, for the decreasing the contamination of *S. aureus and E. coli* O157:H7 in raw meat, hygiene managements from stable to table should be applied. Additionally, it can be advised to the consumer that, raw meat should be cooked adequately before the consumption.

## Authors' Contribution

Study concept and design: NDA, EO, and BO; analysis and interpretationof data: NDA, EO, BO, and GC; drafting of the manuscript: NDA, and GC; critical revision of the manuscript for important intellectual content: NDA and GC.

## Conflict of Interest Disclosures

None.

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## References

1. Dinges MM, Orwin PM, Schlievert PM. Exotoxins of Staphylococcus aureus. Clin Microbiol Rev. 2000;13(1):16-34.
2. Lowy FD. Staphylococcus aureus infections. N Engl J Med. 1998;339(8):520-532. doi:10.1056/NEJM199808203390806.
3. Seo KS, Bohach GA. Staphylococcus aureus. In: Doyle MP, Buchanan RL, eds. Food Microbiology: Fundamentals and Frontiers. 3rd ed. Washington, DC: ASM Press; 2007:493-518.
4. Jay JM, Loessner M, Golden D. Modern food microbiology. 7th ed. New York: Springer Since Business Media; 2005.
5. Normanno G, La Salandra G, Dambrosio A, et al. Occurrence, characterization and antimicrobial resistance of enterotoxigenic Staphylococcus aureus isolated from meat and dairy products. Int J Food Microbiol. 2007;115(3):290-296. doi:10.1016/j.ijfoodmicro.2006.10.049.
6. Mead PS, Slutsker L, Dietz V, et al. Food-related illness and death in the United States. Emerg Infect Dis. 1999;5(5):607-625. doi:10.3201/eid0505.990502.
7. Tauxe RV. Emerging foodborne diseases: an evolving public health challenge. Int J Food Microbiol. 2002;78(1):31-41. doi:10.3201/eid0304.970403.
8. Hodges JR, Kimball AM. The global diet: trade and novel infections. Global Health. 2005;1(1):1-7. doi:10.1186/1744-8603-1-1.
9. Chapman PA, Siddons CA, Cerdan Malo AT, Harkin MA. A 1-year study of Escherichia coli O157 in cattle, sheep, pigs and poultry. Epidemiol Infect. 1997;119(2):245-250.
10. Cray WC, Moon HW. Experimental infection of calves and adult cattle with Escherichia coli O157:H7. Appl Environ Microbiol. 1995;61(4):1586-1590.
11. Arthur TM, Bosilevac JM, Brichta-Harhay DM, Kalchayanand N, Shackelford SD, Wheeler TL, et al. Effects of a minimal hide wash cabinet on the levels and prevalence of Escherichia coli O157: H7 and Salmonella on the hides of beef cattle at slaughter. J J Food Prot. 2007;70(5):1076-9.
12. Elder RO, Keen JE, Siragusa GR, Barkocy-Gallagher GA, Koohmaraie M, Laegreid WW. Correlation of enterohemorrhagic Escherichia coli O157 prevalence in feces, hides, and carcasses of beef cattle during processing. P Proc Natl Acad Sci. 2000;97(7):2999-3003.
13. McEvoy J, Doherty A, Finnerty M, et al. The relationship between hide cleanliness and bacterial numbers on beef carcasses at a commercial abattoir. Lett Appl Microbiol. 2000;30(5):390-395.
14. Omisakin F, MacRae M, Ogden ID, Strachan NJC. Concentration and prevalence of Escherichia coli O157 in cattle feces at slaughter. Appl Environ Microbiol. 2003;69(5):2444-2447.
15. Turkey Statistical Institute. Number of slaughtered animal and quantity of meat production. TUIK website. http://www.tuik.gov.tr/UstMenu.do?metod=temelist. Accessed 2015 September 30, 2015. Published 2015.
16. Mead PS, Griffin PM. Escherichia coli O157: H7. Lancet. 1998;352(9135):1207-1212.
17. Cohen N, Ennaji H, Bouchrif B, Hassar M, Karib H. Comparative study of microbiological quality of raw poultry meat at various seasons and for different slaughtering processes in Casablanca (Morocco). J Appl Poult Res. 2007;16(4):502-508.
18. Gooding CM, Choudary PV. Rapid and sensitive immunomagnetic separation–polymerase chain reaction method for the detection of Escherichia coli O157 [ratio] H7 in raw milk and ice-cream. J Dairy Sci Res. 1997;64(01):87-93.
19. Fantelli K, Stephan R. Prevalence and characteristics of shigatoxin-producing Escherichia coli and Listeria monocytogenes strains isolated from minced meat in Switzerland. Int J Food Microbiol. 2001;70(1):63-69.
20. Cagney C, Crowley H, Duffy G, et al. Prevalence and numbers of Escherichia coli O157: H7 in minced beef and beef burgers from butcher shops and supermarkets in the Republic of Ireland. Food Microbiol. 2004;21(2):203-212.
21. Boerlin P, McEwen SA, Boerlin-Petzold F, Wilson JB, Johnson RP, Gyles CL. Associations between virulence factors of Shiga toxin-producingEscherichia coli and disease in humans. J Clin Microbiol. 1999;37(3):497-503.
22. Law D. Virulence factors of Escherichia coli O157 and other Shiga toxin-producing E. coli. J Appl Microbiol. 2000;88(5):729-745.
23. Fields PI, Blom K, Hughes HJ, Helsel LO, Feng P, Swaminathan B. Molecular characterization of the gene encoding H antigen in Escherichia coli and development of a PCR-restriction fragment length polymorphism test for identification of E. coli O157: H7 and O157: NM. J Clin Microbiol. 1997;35(5):1066-1070.
24. International Organization for Standardization (ISO): Microbiology of food and animal feeding staffs- Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species). 2003; ISO 6888-1.
25. International Organization for Standardization (ISO). Microbiology of food and animal feeding stuffs- Horizontal method for the detection of Escherichia coli O157. 2001; ISO 16654.
26. Sarimehmetoglu B, Aksoy MH, Ayaz ND, Ayaz Y, Kuplulu O, Kaplan YZ. Detection of Escherichia coli O157: H7 in ground beef using immunomagnetic separation and multiplex PCR. Food Cont. 2009;20(4):357-61.
27. Fratamico PM, Bagi LK, Pepe T. A multiplex polymerase chain reaction assay for rapid detection and identification of Escherichia coli O157: H7 in foods and bovine feces. J Food Prot. 2000;63(8):1032-1037.
28. Jiang CM, Liu PH, Ding JP, Liu XG, Hu DL, Shinagawa K. Incidence and pollution of enterotoxigenic Staphylococcus aureus in milk, meat and fish in China. Jpn J Food Microbiol. 2001;18:43-47.
29. Kitai S, Shimizu A, Kawano J, et al. Prevalence and

characterization of Staphylococcus aureus and enterotoxigenic Staphylococcus aureus in retail raw chicken meat throughout Japan. J Vet Med Sci. 2005;67(3):269-274.
30. Desmarchelier PM, Higgs GM, Mills L, Sullivan AM, Vanderlinde PB. Incidence of coagulase positive Staphylococcus on beef carcasses in three Australian abattoirs. Int J Food Mıcrobiol. 1999;47(3):221-229.
31. Shimizu A, Naka M, Kawano J. A follow-up survey of Staphylococcus aureus contamination of commercial raw minced meat at supermarkets and characteristics of isolates. Shokuhin Eiseigaku Zasshi J Food Hyg Soc Jpn. 2008;49(4):320-325.
32. Noveir MR, Dogan HB, Halkman AK. A note on Escherichia coli O157: H7 serotype in Turkish meat products. Meat Sci. 2000;56(4):331-335.
33. Heuvelink AE, Zwartkruis-Nahuis JTM, Beumer RR, De Boer E. Occurrence and survival of verocytotoxin-producing Escherichia coli O157 in meats obtained from retail outlets in The Netherlands. J Food Prot. 1999;62(10):1115-1122.
34. Blanco J, Blanco M, Blanco JE, et al. Verotoxin-producing Escherichia coli in Spain: prevalence, serotypes, and virulence genes of O157: H7 and non-O157 VTEC in ruminants, raw beef products, and humans. Exp Biol Med. 2003;228(4):345-351.
35. Chapman P, Siddons C, Cerdan Malo A, Harkin M. A one year study of Escherichia coli O157 in raw beef and lamb products. Epidemiol Infect. 2000;124(02):207-213.
36. Chinen I, Tanaro JD, Miliwebsky E, et al. Isolation and characterization of Escherichia coli O157: H7 from retail meats in Argentina. J Food Prot. 2001;64(9):1346-1351.
37. Samadpour M, Kubler M, Buck FC, et al. Prevalence of Shiga toxin-producing Escherichia coli in ground beef and cattle feces from King County, Washington. J Food Prot. 2002;65(8):1322-1325.
38. Reinstein S, Fox JT, Shi X, Alam MJ, Renter DG, Nagaraja TG. Prevalence of Escherichia coli O157: H7 in organically and naturally raised beef cattle. Appl Environ Microbiol. 2009;75(16):5421-5423. doi:10.1128/AEM.00459-09.
39. Kalantar E, Alikhani MY, Naseri MH, Torabi V. Antibiotic resistance patterns of STEC and ETEC strains: a study on frozen foods of animal origin and children with acute diarrhea. J Microbiol Infect Dis. 2013;3(01):31-35.
40. Tarr PI, Gordon CA, Chandler WL. Shiga-toxin-producing Escherichia coli and haemolytic uraemic syndrome. Lancet. 2005;365(9464):1073-1086.
41. Vernozy-Rozand C, Ray-Gueniot S, Ragot C, et al. Prevalence of Escherichia coli O157: H7 in industrial minced beef. Lett Appl Microbiol. 2002;35(1):7-11.
42. Carney E, O'Brien SB, Sheridan JJ, McDowell DA, Blair IS, Duffy G. Prevalence and level of Escherichia coli O157 on beef trimmings, carcasses and boned head meat at a beef slaughter plant. Food Microbiol. 2006;23(1):52-59.
43. Karch H, Meyer T, Rüssmann H, Heesemann J. Frequent loss of Shiga-like toxin genes in clinical isolates of Escherichia coli upon subcultivation. Infect Immun. 1992;60(8):3464-3467.
44. Badouei MA, Salehi TZ, Khorasgani MR, Tadjbakhsh H, Brujeni GN. Occurrence and characterisation of enterohaemorrhagic Escherichia coli isolates from diarrhoeic calves. Comp Clin Pathol. 2010;19(3):295-300. doi:10.1016/j.fm.2004.12.001.
45. Koochakzadeh A, Badouei MA, Mazandarani E, Madadgar O. Survey on O157: H7 enterohemorrhagic Escherichia coli (EHEC) in cattle in Golestan province, Iran. Iran J Microbiol. 2014;6(4):276.