Kocatepe Veterinary Journal

Kocatepe Vet J (2018) 11(1): 11-17
DOI: 10.5578/kvj.66163
Submittion: 12.10.2017
Accepted: 04.12.2017

RESEARCH ARTICLE

First Report of *Escherichia coli* O157 From Traditional Turkish Dairy Product Kaymak[#]

Didem SAĞLAM[1], Esra ŞEKER[2*]

[1]Mahfiruz Hatice Sultan Dormitory for Female Students, Republic of Turkey Higher Education Credit and Hostels Institution, Afyonkarahisar, Turkey
[2]Department of Microbiology, Faculty of Veterinary Medicine, Afyon Kocatepe University, Afyonkarahisar, Turkey

[#] This study was supported by the Afyon Kocatepe University Scientific Research Projects Coordination Unit (Grand number 15.SAĞ.BİL.10) and was summarized from the master thesis of first author.

***Corresponding author e-mail:** esraseker@hotmail.com

ABSTRACT

The aim of this study was to isolate the *E. coli* O157:H7/H- serotype from traditional Turkish dairy product kaymak sold in Afyonkarahisar, investigate the *Stx1, Stx2, ehlyA* and *eaeA* genes in isolated strains by PCR and determine the antibiotic resistance of strains to some antibiotics commonly used in Turkey. For this purpose, a total of 100 kaymak samples sold in public bazaars located center town and villages of Afyonkarahisar were collected. *E. coli* O157 was isolated from 3 of 100 samples by using conventional culture methods and serological confirmation tests. The presence of *Stx1, Stx2, ehlyA* and *eaeA* genes in the isolated 3 strains was investigated by PCR. While all of 3 strains harboured the *Stx1* and *ehlyA* genes, the *Stx2* and *eaeA* genes were not found in the strains. The antibiotic resistance of strains was investigated by Kirby-Bauer disc diffusion test. While all of strains were resistant to cephazoline, cefoxitin and ceftiofur, the resistance was also determined in the strains to ampicillin (66.7%), cephalothin (66.7%), ceftriaxone (33.3%), nalidixic acid (33.3%) and trimethoprim/sulphamethoxazole (33.3%). The present study is the first investigation showing the presence of *E. coli* O157 and its virulence genes in the kaymak in Turkey.

Key words: *E. coli* O157:H7, enterohemolysin, intimin, kaymak (clotted cream), Shiga toxin

Geleneksel Türk Süt Ürünü Kaymaktan *Escherichia coli* O157'nin İlk Bildirimi

ÖZ

Bu çalışmada, Afyonkarahisar'da satılan geleneksel bir Türk süt ürünü olan kaymaktan *E. coli* O157:H7/H- serotipinin izolasyonu, izole edilen suşlarda *Stx1, Stx2, ehlyA* ve *eaeA* genlerinin PZR ile araştırılması ve suşların Türkiye'de yaygın olarak kullanılan bazı antibiyotiklere karşı antibiyotik dirençliliğinin belirlenmesi amaçlandı. Bu amaçla, Afyonkarahisar'ın merkez ve köylerindeki halk pazarlarında satılan toplam 100 kaymak örneği toplandı. Konvansiyonel kültür yöntemleri ve serolojik doğrulama testleri kullanılarak 100 kaymak örneğinin 3'ünden *E. coli* O157 izole edildi. İzole edilen 3 suşta *Stx1, Stx2, ehlyA* ve *eaeA* genlerinin varlığı PZR ile araştırıldı. Üç suşun tamamı *Stx1* ve *ehlyA* genlerine sahipken, suşlarda *Stx2* ve *eaeA* genleri bulunmadı. Suşların antibiyotik dirençliliği Kirby-Bauer disk difüzyon testi kullanılarak araştırıldı. Suşların tamamı sefazolin, sefoksitin ve seftiofura dirençli iken, suşlarda ayrıca ampisilin (%66,7), sefalothin (%66,7), seftriakson (%33,3), nalidiksik asit (%33,3) ve trimetoprim/sulfametoksazole (%33,3) karşı da direnç tespit edildi. Sunulan çalışma, Türkiye'de kaymaklarda *E. coli* O157'nin ve serotipin virulens genlerinin varlığını gösteren ilk araştırmadır.

Anahtar kelimeler: *E. coli* O157:H7, enterohemolizin, intimin, kaymak, Şiga toksin

To cite this article: Sağlam D. Şeker E First Report of Escherichia coli O157 From Traditional Turkish Dairy Product Kaymak. Kocatepe Vet J. (2018) 11(1): 11-17..

INTRODUCTION

Escherichia coli O157:H7/H- is a significant pathogen associated with hemorrhagic colitis (HC), hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) in human. This serotype has been known to be one of the most important pathogens of food-borne infections worldwide, posing a great challenge to public health (Karmali 2010, Ko et al. 2016). The primer reservoirs of this bacterial agent are domestic ruminants and the consumption of food products of ruminant origin is associated with serious epidemic outbreaks (Chapman et al. 1993, Upton and Coia 1994, Kiranmayi et al. 2010).

The primer virulence factors of this serotype contributing to the infection pathogenesis are the Shiga toxins, intimin and the plasmid-encoded enterohemolysin (Karmali 2010, Bergan et al. 2012, Ko et al. 2016). Although antimicrobial agents are commonly used for the treatment of several bacterial infections, their usage for treating *E. coli* O157:H7/H- infections are controversial among the researchers (Ikeda et al. 1999, Wong et al. 2000).

According to Turkish Food Codex Communique on Cream and Turkish Cream, kaymak is described to be cream containing at least 60% milk fat, while Afyon kaymak is defined as a product obtained from the boiling of buffalo milk according to the technique and keeping at least 2 minutes at 92 °C, and cooling according to the technique (TGK 2003). This traditional Turkish product has an important marketspace in terms of both the manufacture region and the country. Generally, pure Anatolian buffalo milk is preferred for the production of kaymak because of its high fat content, white color, rich aroma and flavor. If the buffalo milk is insufficient, the cow milk enriched by adding to certain amount of cream can also be used. However, recently, the most of kaymak sold on market and bazaars is originated from cow milk and/or cow and buffalo milk (mix). In the production of traditional kaymak pre-heating is firstly applied to the evening milk. For this purpose, the evening milk is slowly heated at 95 °C for 30 min, cooled and then left at room temperature overnight. The next morning the edge of container is perforated by using a pin, the releasing of milk cream layer is provided and morning milk is added from this perforation. The mixture of milk is slowly heated at 95 °C for 45 min again and allowed to cool at room temperature. After this period, it is refrigerated until morning and the kaymak layer is separated by cutting the next day (Siriken and Erol 2009, Şenel 2010).

Afyonkarahisar province is the famous city of Turkey in terms of traditional dairy products, especially kaymak, produced by buffalo milk. Generally, the researches on Afyon kaymak or kaymak have focused on the microbiological and chemical quality of its (Siriken and Erol 2009, Şenel 2010), but in these studies, the presence of specific bacteria and limited chemical parameters has been investigated. However, sufficient data have not been described concerning the isolation and detection of virulence genes of *E. coli* O157:H7/H- serotype from kaymak samples in Turkey. Therefore, the aim of this study was to investigate the presence of this serotype and its major virulence genes in kaymak and determine the antibiotic resistance of isolated strains to some antibiotics commonly used in Turkey.

MATERIALS and METHODS

Kaymak samples

The present study was carried out on homemade and commercial kaymak samples sold in public bazaars located in center town and villages of Afyonkarahisar province of Western Turkey. For this purpose, a total of 100 kaymak samples (n= 69 homemade, n= 31 commercial) were bought from several bazaars between February and April 2016. Samples were immediately transported to microbiology laboratory in a cool box on ice. Of 100 samples, 57, 32 and 11 were originated from cow milk, cow and buffalo milk (mix) and buffalo milk, respectively.

Isolation and identification of E. coli O157:H7/H- from kaymak samples

Each kaymak sample was aseptically homogenized by mixing in its own plastic container. Ten grams samples were transferred into 90 mL modified Tryptone Soy Broth (mTSB, Oxoid Ltd., UK) containing Novobiocin (20 mg/L) (Oxoid Ltd., UK) and homogenized by vortexing at 120 rpm for 5 min. The mixture was aerobically incubated at 37 °C for 24 hours. A 10 µL aliquot was taken from mTSB and inoculated onto Cefixime-Tellurite added Sorbitol MacConkey agar (CT-SMAC, Oxoid Ltd., UK). The plates were incubated at 37 °C under aerobic conditions for 24 hours. The colourless colonies growing on CT-SMAC agar were evaluated to be suspect and the identification of these colonies was made using Gram staining, oxidase, indole, methyl red, Voges Prouskauer, urease, hydrogen sulphide, citrate, glucose, sucrose, lactose, cellobiose and motility tests. For serological confirmation, *E. coli* O157 latex test kit (Oxoid Ltd., UK) and H7 antisera (Denka Seiken Co. Ltd., Japan) were used (Şeker and Yardımcı 2008). In all applications, EHEC O157:H7 strain EDL 933 and *E. coli* ATCC 25922 were used as the positive and negative control strains, respectively.

Detection of Stx1, Stx2, ehlyA and eaeA genes in E. coli O157:H7/H- strains by PCR

DNAs were extracted from the positive control, negative control and all test strains using boiling method. For this purpose, control and test strains were inoculated onto Trypticase Soy Agar (Oxoid Ltd., UK). After the incubation, one colony was suspended in 500 µL of DNase-RNase free DEPC-treated water and the suspension was subjected to boiling at 100 ºC in a water bath for 10 minutes. Afterwards the suspension was centrifuged at 9,167 *g* for 5 minutes and the supernatant containing bacterial DNA was stored at -20 ºC for PCR mixture.

The primers designed by Otawa et al. (2004) and Osek (2003) were preferred for the detection of *Stx1, Stx2* genes and *ehlyA*, *eaeA* genes, respectively (Table 1). Singleplex PCR was used for the detection of *eaeA* gene, while multiplex PCR (mPCR) was performed for the detection of *Stx1, Stx2* and *ehlyA* genes. PCR mixture used in the present study was as follows: two µL of the extracted DNA were added to 50 µL of PCR mixture of 10X PCR buffer, 2.5 mmol/L $MgCl_2$, 0.2 mmol/L of each dNTP, 2 U Taq polymerase, 0.25 µmol/L of each primer and deionized water. The mPCR amplification conditions of *Stx1, Stx2* and *ehlyA* genes consisted of an initial denaturation step at 95 °C for 4 min, and 30 cycles of 95 °C for 30 s, 57 °C for 30 s, 72 °C for 30 s and a final step at 72 °C for 7 min. The amplification cycles of *eaeA* gene were programmed as 4 min at 95 °C for initial denaturation; 30 cycles, 30 s at 95 °C, 1 min at 45 °C, 1 min at 72 °C; and 7 min final extension step at 72 °C (Şeker et al. 2010). All PCR products were separated by electrophoresis in a 1.5% agarose gel and visualized using ethidium bromide on UV transilluminator. Product sizes were determined by comparison with 100-bp molecular marker (DNA ladder; Fermentas, Vilnius, Lithuania).

Antibiotic susceptibility test

The antimicrobial resistance of positive and negative control strains and test strains was determined by using Kirby-Bauer disc diffusion method on Mueller Hinton agar (Oxoid Ltd., UK) according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI 2013). For this purpose, amoxicillin+clavulanic acid (30µg), ampicillin (10µg), cephalothin (30µg), cephazolin (30µg), cefoxitin (30µg), ceftiofur (30µg), ceftriaxone (30µg), enrofloxacin (5µg), gentamicin (10µg), kanamycin (30µg), streptomycin (10µg), ciprofloxacin (5µg), nalidixic acid (30µg), tetracycline (30µg), imipenem (10µg) and trimethoprim+sulfamethoxazole (25µg) antibiotic discs (Oxoid Ltd., UK) were used. The plates were aerobically incubated for at 37 °C 24 hours.

RESULTS

Isolation and identification findings

According to standard biochemical and serological test results, *E. coli* O157 was identified from 3 of 100 kaymak samples. H7 antigen was not determined in the strains. Of isolated strains 1 was homemade and originated from cow milk, while 2 strains were commercial and originated from cow and buffalo milk (mix).

Distribution of virulence genes

All of 3 strains isolated from kaymak samples harboured the *Stx1* and *ehlyA* genes, while the *Stx2* and *eaeA* genes were not found in the strains. Amplification of *Stx1* (210 bp) and *ehlyA* (534 bp) genes in 3 *E. coli* O157 strains was shown in Figure 1.

Antibiotic susceptibility test

According to Kirby-Bauer disc diffusion test results, all of 3 *E. coli* O157 strains were resistant to cephazolin, cefoxitin and ceftiofur, while they are sensitive to enrofloxacin, gentamicin, ciprofloxacin, tetracycline and imipenem. The resistance to ampicillin (66.7%), cephalothin (66.7%), ceftriaxone (33.3%), nalidixic acid (33.3%) and trimethoprim+sulfamethoxazole (33.3%) was also found in the strains. Antibiotic resistance of strains was shown in Table 2.

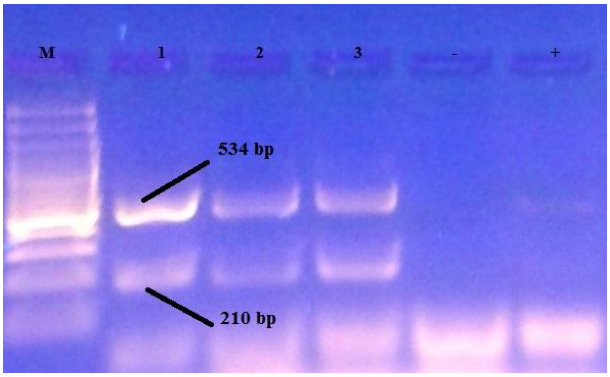

Figure 1: *Stx1* and *ehlyA* mPCR findings in *E. coli* O157 strains. M: 100 bp DNA ladder; lane 1-3: *Stx1* (210 bp) and *ehlyA* (534 bp) genes positive test strains; -: negative control (*E. coli* ATCC 25922); +: positive control (*E. coli* O157:H7 EDL931).

Table 1: Oligonucteotide primers used in this study

Gene		Oligonucleotide sequence (5'–3')	Product size (bp)
Stx1	Forward	TGTAACTGGAAAGGTGGAGTATACA	210
	Reverse	GCTATTCTGAGTCAACGAAAAATAAC	
Stx2	Forward	GTTTTTCTTCGGTATCCTATTCC	484
	Reverse	GATGCATCTCTGGTCATTGTATTAC	
ehlyA	Forward	GCATCATCAAGCGTACGTTCC	534
	Reverse	AATGAGCCAAGCTGGTTAAGCT	
eaeA	Forward	GGGATCGATTACCGTCAT	837
	Reverse	TTTATCAGCCTTAATCTC	

Table 2: Antibiotic resistance of *E. coli* O157 strains

Antibiotic	*E. coli* O157 strains (n=3)					
	S		I		R	
	n	%	n	%	n	%
Amoxicillin+clavulanic acid (30µg)	2	66.7	1	33.3	-	0
Ampicillin (10µg)	1	33.3	-	0	2	66.7
Cephalothin (30µg)	-	0	1	33.3	2	66.7
Cephazolin (30µg)	-	0	-	0	3	100
Cefoxitin (30µg)	-	0	-	0	3	100
Ceftiofur (30µg)	-	0	-	0	3	100
Ceftriaxone (30µg)	2	66.7	-	0	1	33.3
Enrofloxacin (5µg)	3	100	-	0	-	0
Gentamicin (10µg)	3	100	-	0	-	0
Kanamycin (30µg)	2	66.7	1	33.3	-	0
Streptomycin (10µg)	1	33.3	2	66.7	-	0
Ciprofloxacin (5µg)	3	100	-	0	-	0
Nalidixic acid (30µg)	2	66.7	-	0	1	33.3
Tetracycline (30µg)	3	100	-	0	-	0
Imipenem (10µg)	3	100	-	0	-	0
Trimethoprim+sulfamethoxazole (25µg)	2	66.7	-	0	1	33.3

S: Sensitive; I: Intermediate; R: Resistant

DISCUSSION

The present study investigated the presence of *E. coli* O157:H7/H- serotype and its major virulence genes in the kaymak, a traditional Turkish dairy product, and the antibiotic resistance of isolated strains to some antibiotics commonly used in Turkey.

Several researchers have shown the presence of *E. coli* O157:H7/H- in various raw or treated milk and other dairy products of animal origin and emphasized the importance of this serotype in

terms of public health (Şeker and Yardımcı 2008, Rantsiou et al. 2012, Sancak et al. 2015, Tanzifi et al. 2015, Douëllou et al. 2016). Although kaymak is a sought-after Turkish dairy product, only one study investigating the presence of *E. coli* O157 in kaymak has been found in Turkey. İpekcioğlu (2009) reported that *E. coli* O157:H7/H- was detected in none of the examined 100 kaymak samples. In our study, 100 kaymak sold in public bazaars located center town and villages of Afyonkarahisar were examined for the presence of *E. coli* O157:H7/H-. Of the 100 examined samples, 3 were positive for *E. coli* O157. This is the first report of *E. coli* O157 from kaymak in Turkey. Epidemiological data obtained from food-borne outbreaks or individual cases indicate that the contamination of milk and other dairy products by *E. coli* O157:H7/H- may be associated with the external contamination of teats and mastitis, poor milking hygiene, insufficient pasteurization and/or post processing contamination (Şeker and Yardımcı 2008, Farrokh et al. 2013, Sancak et al. 2015; Nobili et al. 2016). In the studies related to the development of this serotype in different environments have been emphasized that *E. coli* O157:H7/H- is more sensitive than *Salmonella* spp. to high temperature values (Berry and Cutter 2000). Heating procedures is applied twice in the production of traditional kaymak; in light of that fact the isolation of this serotype from our samples may be associated with defective pasteurization, the fecal contamination of product and/or poor hygiene after the production.

The pathogenicity of *E. coli* O157:H7/H- is associated with various virulence factors such as Shiga toxins, enterohemolysin and intimin (Karmali 2010, Bergan et al. 2012). Shiga toxins cause microvascular changes *in vivo* and are cytotoxic for selected cell lines *in vitro*, are known to be associated with HC, HUS and bloody diarrhea (Bergan et al. 2012). Although the influence of enterohemolysin produced by EHEC in the pathogenesis remains unclear, it has been considered that enterohemolysins may be used as the epidemiological marker for Shiga toxin-producing *E. coli* (STEC) strains (Farrokh et al. 2013). Another virulence factor intimin is responsible for the formation of attaching/effacing (A/E) lesions on intestinal epithelial cells (Karmali 2010). The low and high prevalence rates of genes encoding these virulence factors have been reported in *E. coli* O157:H7/H- strains isolated from milk and dairy products of cow and buffalo origin (Rahimi et al. 2012, Rantsiou et al. 2012, Douëllou et al. 2016, Nobili et al. 2016). Generally, it was shown that the prevalence of *Stx2* gene was higher than *Stx1* gene and *ehlyA* gene was the most common virulence gene in the strains. Many researchers also emphasized that *eaeA* gene had high prevalence in the plurality of isolate number, while the absence of this gene pointed out in the paucity of isolates (Momtaz et al. 2012, Rantsiou et al. 2012, Douëllou et al. 2016, Nobili et al. 2016). In our study, all of 3 *E. coli* O157 strains harboured the *Stx1* and *ehlyA* genes (Figure 1), while the *Stx2* and *eaeA* genes were not found in the strains. Friedrich et al. (2002) reported that *Stx2* is clinically the most important *Stx* type and the probability of HUS development in infections from strains harbouring *Stx2* is higher than that of strains containing either *Stx1* or both *Stx1* and *Stx2*. However, some researchers emphasized the *Stx1*-producing and/or no Shiga toxin producing *E. coli* O157:H7/H- strains can also pose a potential risk for public health (Schmidt et al. 1999, Friedrich et al. 2002, Bergan et al. 2012). Similarly, numerous investigators underlined the strong association between the carriage of *eaeA* gene and the capacity of STEC to cause severe human disease, especially HUS (Beutin et al. 2004, Karmali 2010). Nevertheless, it was reported its production was not essential for pathogenesis, because a number of sporadic cases of HUS were caused by *eaeA*-negative strains (Karch et al. 2005). Geographical variations, the difference of tested material and the deficiency of strain number isolated in this study may be the reason for discrepancy in the distribution of virulence genes compared to results of other studies.

Antimicrobial resistance has been recognized as a global health problem for many decades and several programs have been set up for its surveillance in human and veterinary medicine. Some of the antibiotic resistance genes identified in food bacteria have also been identified in humans, providing indirect evidence for transfer by food handling and/or consumption (Founou et al. 2016). The use of antibiotics in the treatment of *E. coli* O157:H7/H- infections is controversial. Some authors reported that antimicrobials may lyse bacterial cell walls, thereby liberating Shiga toxins and/or cause increased expression of Shiga toxin genes *in vivo* (Wong et al. 2000), while others suggested that some antibiotics applied in the early period of infection may prevent disease progression to HUS and HC because of the susceptibility of many STEC O157:H7/H- isolates to numerous antimicrobials (Ikeda et al. 1999). In the investigations related to antibiotic resistance of *E. coli* O157:H7/H- strains isolated from milk and dairy products, the researchers emphasized the isolates showed the high resistance rates and/or multiple drug resistance (Momtaz et al. 2012, Rahimi et al. 2012, Reuben et al. 2013). In this study, the antibiotic resistance of 3 *E. coli* O157 strains isolated from traditional kaymak samples to 16 different antibiotics commonly used in Turkey was investigated by Kirby-Bauer disc diffusion test.

All of tested strains were resistant to cephazoline, cefoxitin and ceftiofur, followed by ampicillin (66.7%), cephalothin (66.7%), ceftriaxone (33.3%), nalidixic acid (33.3%) and trimethoprim+sulfamethoxazole (33.3%) (Table 2). However, the resistance rates obtained from our study were insufficient to comment the resistance profiles of strains because of the low isolate number.

CONCLUSIONS

In conclusion, the presence of *E. coli* O157 serotype and its major virulence genes in traditional kaymak was reported for the first time in Turkey. Afyonkarahisar located in Western Turkey has critical position in the breeding of dairy animals, especially water buffaloes, and the kaymak obtained from these animals have an important consumer portion in Turkey. Although an outbreak connected to *E. coli* O157:H7/H$^-$ has not been reported in Turkey so far, there is a need for awareness that kaymak, like other foods of animal origin, can pose a potential risk of *E. coli* O157 infections in human. Also, the data deficiency on the presence of this zoonotic pathogen in traditional kaymak in Turkey should be eliminated through with future investigations.

REFERENCES

Karmali MA, Gannon V, Sargeant JM. Verocytotoxin-producing *Escherichia coli* (VTEC). Vet Microbiol. 2010; 140: 360-370.

Ko H, Maymani H, Rojas-Hernandez C. Hemolytic uremic syndrome associated with *Escherichia coli* O157:H7 infection in older adults: a case report and review of the literature. J Med Case Rep. 2016; 10: 1-4.

Chapman PA, Siddons CA, Wright DJ, Norman P, Fox J, Crick E. Cattle as a possible source of verocytotoxin-producing *Escherichia coli* O157 infections in man. Epidemiol Infect. 1993; 111: 439-447.

Upton P, Coia J. Outbreak of *Escherichia coli* O157 infection associated with pasteurised milk supply. Lancet. 1994; 344: 1015.

Kiranmayi CB, Krishnaiah N, Mallika EN. *Escherichia coli* O157:H7-An emerging pathogen in foods of animal origin. Vet World. 2010; 3: 382-389.

Bergan J, Lingelem ABD, Simm R, Skotland T, Sandvig K. Shiga toxins. Toxicon. 2012; 60: 1085-1107.

Ikeda K, Ida O, Kimoto K, Takatorige T, Nakanishi N, Tatara K. Effect of early fosfomycin treatment on prevention of hemolytic uremic syndrome accompanying *Escherichia coli* O157:H7 infection. Clin Nephrol. 1999; 52: 357-362.

Wong CS, Jelacic S, Habeeb RL, Watkins SL, Tarr PI. The risk of hemolytic-uremic syndrome after antibiotic treatment of *Escherichia coli* O157:H7 infections. New Eng J Med. 2000; 342: 1930-1936.

TGK. Türk Gıda Kodeksi. Türk Gıda Kodeksi-Krema Ve Kaymak Tebliği. Tebliğ No: 2003/34, R.G. Tarihi: 27.09.2003 R.G. Sayısı: 25242. 2003. (Erişim Tarihi: 12.03.2016).

Siriken B., Erol I. Microbiological and chemical quality of Afyon clotted cream. J Anim Vet Adv. 2009; 8: 2022-2026.

Şenel E. Some carbonyl compounds and free fatty acid composition of Afyon Kaymagı (clotted cream) and their effects on aroma and flavor. Grasas Aceites. 2011; 62: 418-427.

Şeker E, Yardımcı H. First isolation of *Escherichia coli* O157:H7 from faecal and milk specimens from Anatolian water buffaloes (*Bubalus bubalus*) in Turkey. J S Afr Vet Assoc. 2008; 79: 167-170.

Otawa K, Sato M, Sasaki T, Sasaki H, Nonaka J, Ito K, Kuroki T, Nakai Y. Genetic analysis of shiga-toxigenic *Escherichia coli* isolates from cattle in a limited region. Anim Sci J. 2004; 75: 261-269.

Osek J. Development of a multiplex PCR approach for the identification of shiga toxin-producing *Escherichia coli* strains and their major virulence factor genes. J Appl Microbiol. 2003; 95: 1217-1125.

Şeker E, Kuyucuoğlu Y, Sareyyüpoğlu B, Yardımcı H. PCR detection of Shiga toxins, enterohaemolysin and intimin virulence genes of *Escherichia coli* O157:H7 strains isolated from faeces of Anatolian Water Buffaloes in Turkey. Zoonoses Public Health. 2010; 57: e33–e37.

CLSI. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. Twenty Third informational supplement. CLSI document M100-S23. Clinical and Laboratory Standards Institute, Wayne, PA, USA, 2013.

Rantsiou K, Alessandria V, Cocolin L. Prevalence of shiga toxin-producing *Escherichia coli* in food products of animal origin as determined by molecular methods. Int J Food Microbiol. 2012; 154: 37-43.

Sancak YC, Sancak H, Isleyici O, Durmaz H. Presence of *Escherichia coli* O157 and O157:H7 in raw milk and Van herby cheese. Bull Vet Inst Pulawy. 2015; 59: 511-514.

Tanzifi P, Bahrami AR, Rahimi E. Study on antimicrobial resistance of *Escherichia coli* O157:H7/NM isolated from raw bovine, camel, water buffalo, caprine and ovine milk. Res J Recent Sci. 2015; 4: 20-22.

Douëllou T, Delannoy S, Ganet S, Mariani-Kurkdjian P, Fach P, Loukiadis E, Montel M, Thevenot-Sergennet D. Shiga toxin-producing *Escherichia coli* strains isolated from dairy products - Genetic diversity and virulence gene profiles. Int J Food Microbiol. 2016; 232: 52-62.

İpekçioğlu V. Searching for some pathogenic bacteria in the Afyon kaymagi supplied to the market in Afyonkarahisar. MSc Thesis, University of Afyon Kocatepe, Institute of Health Sciences, Afyonkarahisar, Turkey, 2009.

Farrokh C, Jordan K, Auvray F, Glass K, Oppegaard H, Raynaud S, Thevenot D, Condron R, de Reu K, Govaris A, Heggum K, Heyndrickx M, Hummerjohann J, Lindsay D, Miszczycha S, Moussiegt S, Verstraete K, Cerf O. Review of shiga-toxin-producing *Escherichia coli* (STEC) and their significance in dairy production. Int J Food Microbiol. 2013; 162: 190-212.

Nobili G, Franconieri I, Basanisi MG, La Bella G, Tozzoli R, Caprioli A, La Salandra G. Short communication: Isolation of Shiga toxin-producing *Escherichia coli* in raw milk and mozzarella cheese in southern Italy. J Dairy Sci. 2016; 99: 7877-7780.

Berry ED, Cutter CN. Effect of acid adaptation of *Escherichia coli* O157:H7 on efficacy of acetic acid spray washes to decontaminate beef carcass tissue. Appl Environ Microbiol. 2000; 66: 1493-1498.

Rahimi E, Momtaz H, Anari MMH, Alimoradi M, Momeni M, Riahi M. Isolation and genomic characterization of *Escherichia coli* O157:NM and *Escherichia coli* O157:H7 in minced meat and some traditional dairy products in Iran. Afr J Biotechnol. 2012; 11: 2328-2332.

Momtaz H, Farzan R, Rahimi E, Dehkordi FS, Souod N. Molecular characterization of Shiga toxin-producing *Escherichia coli* isolated from ruminant and donkey raw milk samples and traditional dairy products in Iran. Sci World J. 2012; 2012: 231342.

Friedrich AW, Bielaszewska M, Zhang WL, Pulz M, Kuczius T, Ammon A, Karch H. *Escherichia coli* harboring Shiga toxin 2 gene variants: frequency and association with clinical symptoms. J Infect Dis. 2002; 185: 74-84.

Schmidt H, Scheef J, Huppertz HI, Frosch M, Karch H. *Escherichia coli* O157:H7 and O157:H- strains that do not produce Shiga toxin: phenotypic and genetic characterization of isolates associated with diarrhea and hemolytic-uremic syndrome. J Clin Microbiol. 1999; 37: 3491-3496.

Beutin L, Krause G, Zimmermann S, Kaulfuss S, Gleier K. Characterization of shiga toxin-producing *Escherichia coli* strains isolated from human patients in Germany over a 3-year period. J Clin Microbiol. 2004; 42: 1099-1108.

Karch H, Tarr PI, Bielaszewska M. Enterohaemorrhagic *Escherichia coli* in human medicine. Int J Med Microbiol. 2005; 295: 405-418.

Founou LL, Founou RC, Essack SY. Antibiotic resistance in the food chain: a developing country-perspective. Front Microbiol. 2016; 7: 1881.

Reuben RC, Okolocha EC, Bello M, Tanimu H. Occurrence and antibiogram of *Escherichia coli* O157:H7 in locally fermented milk (Nono) sold under market conditions in Nasarawa State, Nigeria. Int J Sci Res. 2013; 2: 591-598.