COMPARISON OF PREVALENCE AND GENETIC DIVERSITY OF ESCHERICHIA COLI O157:H7 IN CATTLE AND SHEEP

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ABSTRACT
In this study the prevalence of Escherichia coli O157:H7 was detected by immunomagnetic separation (IMS) based cultivation technique and polymerase chain reaction (PCR) in feces and/or colon tissue of cattle (n= 282) and sheep (n= 218) at slaughterhouse. The major virulence genes, intimin variants, Shiga toxin variants and antibiotic resistance genes of the isolates were examined by PCR and genomic diversity of the cattle and sheep E. coli O157:H7 isolates were assessed using pulsed field gel electrophoresis (PFGE). In the present study the prevalence of E.coli O157:H7 was found higher in sheep (6.4 %) than in cattle (3.9 %). All the E.coli O157:H7 isolates were detected as positive for at least one stx gene and positive for other virulence genes. Twelve (29.3 %) and one (2.4 %) of the cattle isolates carried stx1 and stx2 gene, respectively. However 11 (17.7 %) of the sheep E. coli O157:H7 isolates carried stx1 and five (8.1 %) of the isolates harbored stx2 gene only. At least one antibiotic resistance gene was detected from 35 isolates. E. coli O157:H7 isolates from four sheep and three cattle harbored tetB gene. From three cattle and one sheep samples stx1 carrying E. coli O157:H7 were isolated. Among them, isolates from 2 cattle and one sheep samples were carried both tetB and strA. Isolates were grouped into six different clusters. From a cattle and a sheep, two different E. coli O157:H7 which have different PFGE patterns, were isolated. It can be concluded that sheep pose a risk as cattle for STEC O157:H7 contamination in Turkey.

Keywords: E. coli O157:H7; virulence genes; stx variants; antibiotic resistance; PFGE

INTRODUCTION
Escherichia coli O157:H7 has emerged as a pathogen of considerable public health importance which causes a spectrum of illnesses ranging from hemorrhagic colitis to hemolytic-uremic syndrome (HUS) worldwide. Gastrointestinal system of the ruminants is the primary reservoir of this organism (Meng et al., 2001). Many studies showed that both dairy and beef cattle were the primary source and the carrier of the bacterium (Chapman et al., 1993; Byrne et al., 2003; Shin et al., 2014). Limited number of studies reported sheep as a reservoir of this emerging pathogen (Söderlund et al., 2012). Several virulence factors have been described in E. coli O157:H7, the major ones are known as Shiga toxins (stx1 and stx2) which are responsible for life threatening illnesses like HC and HUS. E. coli O157:H7 produces putative virulence genes such as intimin (encoded by eaeA) which is necessary for attaching and efacing adhesion on host cell membrane, and hemolysin (encoded by hly). There are several intimin variants identified and they were shown to affect tissue tropism and colonization site (Mundy et al., 2007). In addition, espA (E. coli secreted protein A) and lpf (Long polar fimbria) are the virulence genes which are important in tropism, attachment, persistence and virulence of E. coli O157:H7, were identified in recent years (Torres et al., 2009).

Antibiotic treatment in E. coli O157:H7 infections in human is not always possible because of lysis of the cells and increased expression and release of the Shiga toxins (Stx) in the intestinal tract (Wong et al., 2000). However using some antimicrobials in the early stage of infection may be protective against hemolytic uremic syndrome (HUS) progression (Ikeda et al., 1999). Due to extensive use of antibiotics in veterinary medicine for prophylaxis or growth promotion in animal production in several studies, resistant E. coli O157:H7 strains were reported to various antibiotics (Schroeder et al., 2004; Goncuoglu et al., 2010). In a report 79.8% of the E. coli O157:H7 isolates were found to carry one or more antibiotic resistance genes (Sriniwasan et al., 2007).

The objectives of this study were to determine the prevalence of Escherichia coli O157:H7 by immunomagnetic separation (IMS) based cultivation technique and PCR in feces and/or colon tissue of cattle and sheep, to detect the major virulence genes, intimin variants, Shiga toxin variants and antibiotic resistance genes of the isolates by polymerase chain reaction (PCR) and to determine the genomic diversity using pulsed field gel electrophoresis (PFGE).

MATERIAL AND METHODS
Sample collection
A total of 282 cattle consisting 207 beef and 75 dairy cattle and 218 sheep (with a total of 500 animals) feces and/or colon tissue samples were collected from a slaughterhouse within 28 visits in Ankara province. Samples were taken into sterile filtered bags and taken into laboratory in an ice box and analyzed within 2 hours.

Microbiological analysis
IMS based cultivation technique was used for the isolation of E. coli O157 (Byrne et al., 2003). Ten grams of samples were weighed to a sterile bag and suspended with 90 ml EC broth (Oxoid CM0853, Hampshire, UK) containing novobiocin (20 µg/L; Sigma N-1628, St. Louis, USA) and incubated at 37°C at 100 rpm/min for 18 h in a shaking incubator (Bellox Shel Lab Shaking Incubator S16R, Oregon, USA). Then, IMS was performed with 20 µl of magnetic beads (Dynabeads anti E. coli O157, Dynal, Norway) according to the manufacturer’s protocol.

Following to IMS procedure, 100 µl of resuspended suspension was plated on Cefixime-tellurite (Oxoid SR0172) supplemented Sorbitol MacConkey Agar (Oxoid CM0813) and incubated at 42°C for 24 h. After incubation, sorbitol negative colonies were tested for the O157 antigen by latex agglutination (Oxoid DR0620) and up to five positive colonies were picked for PCR analysis.
PCR analysis for the detection of virulence genes

DNA extraction was performed by Chelex-100 (Bio-Rad, Hercules, CA, USA) resin based technique, using proteinase K (20 mg/ml; Applichem GmbH, Darmstadt, Germany). Virulence genes including: stx1, stx2, eaeA, hly, bfpA, tetA, sulI [Fratamico et al., 2000] were detected by multiplex PCR: espA (McNally et al., 2001) and lpfA1-3 (Torres et al., 2009) genes were detected by PCR. E. coli O157:H7 ATCC 43895 (stx1+, stx2+, eaeA+, hly+, lpfA1-3+, espA+) was used as positive control.

Detection of intimin and Shiga toxin variants of E. coli O157:H7 isolates

Intimin variants α1, α2, β, β1, β2, γ1 and γ2/0 were tested in eaeA gene detected intimin harboring isolates by previously published primer pairs and PCR conditions (Blanco et al., 2004). E. coli O157:H7 ATCC 43895 (eaeA+) was used as positive control.

E. coli O157:H7 isolates were subjected to consecutive multiplex and conventional PCR assays for determination of stx1 variants (stx1, [Zhang et al., 2002] and stx2 [Bürk et al., 2003]) and/or stx2 variants (stx2a, stx2b, stx2c, stx2g [Osek, 2003] and stx2 [Leung et al., 2003]). E. coli O157:H7 ATCC 43895 (stx2c+), strains E. coli O157:NM 13798 (stx2a+), E. coli O62:H 55198 (stx2g+), E. coli O139:K12 10786 (stx2b+), E. coli O3:H18 214/125 (stx2c+) and E. coli O2:H25 S86 (stx2g+) were used as positive controls.

Detection of antibiotic resistance genes by multiplex PCR

Antimicrobial resistance genes encoding for the tetracycline efflux pump (tetA, tetB, tetC, tetD, tetE, and tetG); streptomycin phosphotransferases (strA and strB); aminoglycoside adenylyltransferase (aadA); chloramphenicol transporter nonenzymatic chloramphenicol-resistance protein (cmrA); florfenicol export protein (floR); dihydropteroate synthetase type I (sulI); dihydropteroate synthetase type II (sulII); and beta-lactamase-ampicillin resistance (ampC) in E. coli O157:H7 isolates were determined by PCR according to Srinivasan et al., (2007).

Genomic characterization by PFGE

Escherichia coli O157:H7 isolates were sub-typed by PFGE technique of CHEF electrophoresis described by Harsono et al., (1993). Genomic DNA was digested in agarose plugs with XbaI (Promega) as recommended by the manufacturer. The resulting DNA fragments were resolved by CHEF-PFGE with a CHEF-DR III apparatus (Bio-Rad Laboratories, CA, USA) at 200 V for 19 h at 14°C and switch times from 1 to 60 s. Low-range lambda concatemers (Promega) were used as DNA size standards. The fragments were visualized by a gel documentation system (Syngene Ingenius). GeneTools software (version 3.08.01; Syngene, United Kingdom) was used for processing the gel image. PFGE results were ascertained by the presence, absence and similarity of restriction fragments and the subtypes were coded as A, B, C, D, E and F.

RESULTS AND DISCUSSION

Fourteen of 218 sheep and 11 of 282 cattle were determined as positive for E. coli O157:H7. The prevalence of E.coli O157:H7 was found in sheep as 6.4 % and in cattle as 3.9 %. Among cattle samples, dairy cattle prevalence of E. coli O157:H7 was higher than beef cattle samples with a ratio of 5.3 % and 3.4 %, respectively. Most of the isolates (20/25, 80%) were determined in warm months (spring and summer). E. coli O157:H7 was isolated from 54.5 % (6/11) of all positive cattle samples in the July. Other one and four E. coli O157:H7 positive cattle samples were recovered in May and October, respectively. Seasonal distribution of E. coli O157:H7 is similar in sheep with cattle samples. Ten out of 14 (71.4 %) E. coli O157:H7 positive samples were taken in summer; three positive samples were detected in spring. Only in one (9.1 %) sheep winter sample E. coli O157:H7 was found (Tab. 1).

Table 1 Seasonal distribution of E. coli O157:H7 in cattle and sheep feces and/or colon tissue samples

<table>
<thead>
<tr>
<th>Season</th>
<th>Months</th>
<th>Number of visits</th>
<th>Number of samples</th>
<th>Number of positive samples</th>
<th>% Seasonal distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spring</td>
<td>April</td>
<td>3</td>
<td>5C+9S</td>
<td>2S</td>
<td>6.3%C, 16.7%S</td>
</tr>
<tr>
<td></td>
<td>May</td>
<td>4</td>
<td>11C+9S</td>
<td>1C+1S</td>
<td></td>
</tr>
<tr>
<td></td>
<td>June</td>
<td>5</td>
<td>30C+68S</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Summer</td>
<td>July</td>
<td>6</td>
<td>5C+50S</td>
<td>6C+3S</td>
<td>6.1%C, 5.9%S</td>
</tr>
<tr>
<td></td>
<td>August</td>
<td>4</td>
<td>15C+52S</td>
<td>7S</td>
<td></td>
</tr>
<tr>
<td>Autumn</td>
<td>September</td>
<td>2</td>
<td>25C</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>October</td>
<td>2</td>
<td>36C</td>
<td>4C</td>
<td>6.6%C</td>
</tr>
<tr>
<td></td>
<td>December</td>
<td>3</td>
<td>61C+50S</td>
<td>1S</td>
<td></td>
</tr>
<tr>
<td>Winter</td>
<td>January</td>
<td>1</td>
<td>35C</td>
<td>-</td>
<td>3.3%S</td>
</tr>
<tr>
<td></td>
<td>February</td>
<td>1</td>
<td>10C</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

C: Cattle; S: Sheep

In order to find the E. coli O157:H7 contamination with multiple strains, up to five colonies were picked from the positive samples for further molecular characterization. For this purpose a total of 103 colonies (41 cattle and 62 sheep isolate) were isolated from these positive animals (14 sheep and 11 cattle). All of the 103 colonies harbored at least one stx1 gene and 74 of them (71.8 %) were found to carry both of toxin genes. Additionally, all E. coli O157:H7 isolates harbored hly, eaeA, lpfA, espA genes and harbored eae-γ1 as an intimin variant (Tab 2). When we compared the cattle and sheep isolates, 12 (29.3 %) of the cattle isolates carried stx1 and one (2.4 %) stx2 gene; however only 11 (17.7 %) of the sheep E. coli O157:H7 isolates carried stx1 and five (8.1 %) harbored stx2 gene only. In the study, 28 of 41 (68.3 %) cattle E. coli O157:H7 colonies and 46 of 62 (75.4 %) sheep E. coli O157:H7 colonies harbored stx1 and stx2. Six (5.8 %) and 23 (22.3 %) of 103 isolates carried stx1 and stx2, respectively. In stx1 and stx2 positive isolates stx1 and stx2, variants were detected. In general, colonies that were isolated from the same sample harbored the same toxin profile except a cattle (coded as C3 in Tab 2) and a sheep (coded as S4 in Tab 2) sample. In sample C3 5 E. coli O157:H7 were picked during isolation; although 4 of them had both stx1 and stx2, Shiga toxin genes, one colony had only stx2. Similarly in sample S4, 4 of the colonies had both stx1, and stx2, but one colony had only stx2. This means a sheep and a cattle that we sampled, carried at least two different E. coli O157:H7 strains.

<table>
<thead>
<tr>
<th>Sample no* (number of colonies)</th>
<th>H7</th>
<th>hly</th>
<th>lpf</th>
<th>espA</th>
<th>stx1 variant</th>
<th>stx2 variant</th>
<th>Intimin variant eae-γ1</th>
<th>Antibiotic resistance genes</th>
<th>PFGE group</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1 (5)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>stx1</td>
<td>stx2</td>
<td>+</td>
<td>tetB, strA</td>
<td>A</td>
</tr>
<tr>
<td>S7 (4)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>stx1</td>
<td>stx2</td>
<td>+</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>S8 (4)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>stx1</td>
<td>stx2</td>
<td>+</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>S9 (5)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>stx1</td>
<td>stx2</td>
<td>+</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>S10 (5)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>stx1</td>
<td>stx2</td>
<td>+</td>
<td>tetB, strA</td>
<td>A</td>
</tr>
<tr>
<td>S12 (2)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>stx1</td>
<td>stx2</td>
<td>+</td>
<td>tetB, strA</td>
<td>A</td>
</tr>
<tr>
<td>C4 (4)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>stx1</td>
<td>stx2</td>
<td>+</td>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td>S3 (5)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>stx1</td>
<td>stx2</td>
<td>+</td>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td>S5 (5)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>stx1</td>
<td>stx2</td>
<td>+</td>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td>S13 (4)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>stx1</td>
<td>stx2</td>
<td>+</td>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td>C2 (5)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>stx1</td>
<td>stx2</td>
<td>+</td>
<td>strA</td>
<td>C</td>
</tr>
<tr>
<td>C5 (2)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>stx1</td>
<td>stx2</td>
<td>+</td>
<td>strA</td>
<td>C</td>
</tr>
<tr>
<td>C1 (5)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>stx1</td>
<td>stx2</td>
<td>+</td>
<td>strA</td>
<td>C</td>
</tr>
<tr>
<td>S2 (5)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>stx1</td>
<td>stx2</td>
<td>+</td>
<td>strA</td>
<td>C</td>
</tr>
<tr>
<td>S4 (4)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>stx1</td>
<td>stx2</td>
<td>+</td>
<td>strA</td>
<td>E</td>
</tr>
</tbody>
</table>
Thirty five of 103 isolates (34 %) carried at least one antibiotic resistance gene. 
E. coli O157:H7 isolates from four sheep and three cattle harbored tetB gene. From three cattle and one sheep samples strA carrying E. coli O157:H7 were isolated. Among them, isolates from 2 cattle and one sheep samples were carried both tetB and strA genes. From tetB and strA were the only detected antibiotic resistance genes from the positive samples. Within the 14 antibiotic resistance genes carrying isolates (9 cattle and 5 sheep) both tetB and strA were determined while from 12 (1 cattle and 11 sheep) and nine E. coli O157:H7 (4 cattle and 5 sheep) only tetB or strA were detected, respectively (Tab 2). Representative PCR results are shown in the figure 1.

**Figure 1** Representative PCR gel electrophoresis of virulence genes detected positive control strains and isolates

**Lane M:** 100 bp DNA marker; **Lane 1:** Negative control; **Lane 2:** E. coli O157:H7, ATCC 43895 ( positive-control); **Lane 3:** E. coli O157:H7, ATCC 43895 ( stain 1, stain 2, eaeA, hly); **Lane 4:** E. coli O157:H7, NCTC 12900 ( stain 1, stain 2, eaeA, hly); **Lane 5:** E. coli O157:H7 isolate ( stain 1, stain 2, eaeA, hly); **Lane 6:** E. coli O157:H7 isolate ( stain 1, stain 2, eaeA, hly); **Lanes 7:** E. coli O157:H7 isolate (eae gene); **8:** E. coli O157:H7 isolate (eaeA gene); **9:** E. coli O157:H7 isolate (lpfA1-3 gene); **Lanes 10:** E. coli O157:H7, ATCC 43895 ( stain 1); **Lane 11:** E. coli O157:NM, 137/98 ( stain 1, stain 2); **Lane 12:** E. coli O62:H1, 551/98 ( stain 2); **Lane 13:** E. coli O139:K12, 107/86 ( stain 2); **Lane 14:** E. coli O:K18, 214/125 ( stain 2)

PFGE data indicated that there were six distinct restriction endonuclease digestion profiles (REDP) among the 103 isolates examined and coded as A, B, C, D, E and F (Tab 1). In pattern A, 29 isolates belonged to six sheep (25 isolates) and one cattle (4 isolates). Pattern A isolates were achieved from four different collection visits. Four sheep samples (18 isolates) that were from same collection date showed different toxin profile. Group B isolates (21 isolates) were belonged to five different animals (3 sheep and 2 cattle) and isolates showed quite distinct toxin profiles. Five isolates from one cattle and five isolates from one sheep were taken part in group C and D, respectively. Both isolates harbored two of the toxin genes. In group E, nine (36 %) of the 25 positive cattle (7) and sheep (2) colonies showed the same genomic profile. Group E isolates were recovered from four different visits. A total of 35 isolates (25 from cattle and 10 from sheep) showed different toxin profiles some of them were harbored stx1 and stx2 together (23 isolates). One isolate was carried stx1 while nine were stx2. As it is shown in Table 2, remarkable results of the study were seen in group E. One isolate from cattle (coded as C3) and one from sheep (coded as S4) showed different genomic profiles from others 4 sub-colonies which were isolated the same samples. They had different toxin genes from their positive animal sub-colonies. The isolate of concern from cattle was detected as stx1 and stx2 positive on the other hand other colonies (4 colonies) from the same cattle harbored stx2 only. Also this isolate carried tetB where the rest of the isolates from the same cattle carried strA as an antibiotic resistance gene. Likewise isolate of concern from sheep harbored only stx2 although others (4 colonies) from the same sheep harbored both of the toxin genes together. These results indicated that this cattle and sheep were simultaneously contaminated with two different E. coli O157:H7. Two sheep isolates (8 isolates) were grouped as F and isolates harbored both of the toxin genes. Four isolates from one sheep carried tetB and strA which the isolates from other positive sheep did not.

In this study which is an important data on the presence of E. coli O157:H7 in sheep in Turkey, the prevalence was found as 6.4 %. Lower than our results, 5.4 % of sheep faeces samples in Ethiopia (Mersha et al., 2010) and 1.8 % of the Swedish sheep (Siderlund et al., 2012) were found to carry E. coli O157:H7 while in North Wales in none of the sheep feecal samples this pathogen was detected (Alfelli et al., 2013).

In the present study the prevalence of E.coli O157:H7 was found higher in sheep (6.4 %) than in cattle (3.9 %). (Van Donkersgoed et al., 1999) reported that prevalence of E. coli O157:H7 in fecal samples of cattle at slaughter age in Canada was 7.5 % by IMS. In another study from 1.5 % of fecal and/or tissue samples of healthy cattle in the USA E. coli O157:H7 was isolated (Byrne et al., 2003). Different from the present study only 3 of 1,300 (0.2 %) fecal samples collected from adult cattle using IMS technique in Norway were found to be contaminated with E. coli O157:H7 (Johnsen et al., 2001). In the study, the PFGE analysis revealed two different PFGE profiles among 3 isolates. The toxin profiles between these groups were showed differences such as two isolates in the same group have stx1, eae and fliC, the other isolate from the other group has both stx2 and stx1 with eae and fliC (Hancock et al., 1997).

In the study, the prevalence of E. coli O157:H7 in feces of healthy (3.7 %) and diarrheic cattle (4.3 %) were nearly the same. This can be explained that cattle can carry E. coli O157:H7 without showing any symptoms of disease (Meng et al., 2001). It was found that out of 207 beef cattle, and 75 dairy cattle samples, seven (3.4 %), and four (5.3 %) were found to be contaminated with E. coli O157:H7, respectively. However it is widely believed that dairy herds are the primary reservoirs of E. coli O157:H7 (Hancock et al., 1997).

The seasonal distribution of E. coli O157:H7 were 11.8, 5.9, 6.6 and 0.7 % during the spring, summer, autumn and winter, respectively. Similar to the previous studies (Van Donkersgoed et al., 1999; Johnsen et al., 2001) our results showed that the prevalence of E. coli O157:H7 in cattle and sheep in tested samples was higher in warm months (6.6 %) than in cold months (2.5 %). In the present study, all 27 isolates from 15 sheep and 12 cattle which 25 of them were isolated from different samples and two of them have different toxin profile from the other sub-colonies isolated from same samples, were found to carry at least one toxin gene (stx1 or stx2). Eighteen (18/27; 66.7 %) of the isolates were positive both for stx1 and stx2, seven of them (7/27; 25.9 %) were positive for only Shiga toxin 2 variant stx2 and two of them (2/27; 7.4 %) were positive for alone Shiga toxin 1 variant stx1. Importantly, it was reported that in most of human E. coli O157:H7 cases stx2 gene was more important in generating illness than stx1 gene harboring strains on the epidemiological study (Boerlin et al., 1999). Also in four different REDP, most of the isolates (72.7 %) were shown same toxin profiles in the same genomic groups. In a study performed in Turkey, where differs from our results that 9 of the 13 E. coli O157:H7 isolates from cattle feces harbored only stx2 and 2 of the isolates were found to carry stx1 and stx1 toxin genes (Yilmaz et al., 2006). In a previous study, presence of stx1, stx2 and stx2 variants in E. coli isolated from asymptomatic individuals or patients of clinical manifestations of either HUS or diarrheae without HUS was compared and they found out that the presence of stx1 can more likely cause HUS while presence of stx2 may manifest a milder case (Friedrich et al., 2002). In a different study, a higher in vitro cytotoxicity was also reported for stx2 carrying E. coli O157:H7 than stx1-stx1 or stx1-stx2 carrying strains (Lefebvre et al., 2010). According to these studies it can be concluded that E. coli O157:H7 positive cattle and sheep were contaminated with highly virulent strains and this may pose potential public health risk.

In the present study, among the tested antibiotic resistance genes, only tetB and strA were detected. In a previous study, 25 (26.0 %) of the 96 E. coli O157:H7 cattle isolates harbored at least one antibiotic resistance gene. Twenty six out of 102 E. coli O157:H7 (25.5 %) were carrying one or more tested tetracycline resistance genes. In the study, tetC, tetA and tetB were detected with a ratio of
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