Acta Veterinaria-Beograd 2016, 66 (4), 520-533
UDK: 636.2:579.842.1/.2; 636.32/.38:579.842.1/.2
DOI: 10.1515/acve-2016-0045

Research article

MOLECULAR CHARACTERIZATION OF ESBL-PRODUCING *ESCHERICHIA COLI* ISOLATED FROM HEALTHY CATTLE AND SHEEP

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(Received 28 March; Accepted 26 September 2016)

The present study aims to characterize ESBL-producing *Escherichia coli* isolated from healthy cattle and sheep in the Burdur province of Turkey. Fecal samples from a total of 200 cattle and 200 sheep were tested and ESBL-producing *E. coli* was isolated from 31 (15.5%) cattle and three (1.5%) sheep samples using the Clinical and Laboratory Standards Institute's combined disk method. Among the ESBL gene classes detected by PCR, bla_{CTX-M} was the most frequent type, followed by the bla_{TEM} and bla_{SHV} families. ESBL-producing *E. coli* isolates showed co-resistance to multiple classes of antibiotics including aminoglycosides, phenicols, quinolones, folate pathway inhibitors and tetracyclines. The resistance rates were higher in the cattle isolates than in the sheep isolates. Phylogenetic grouping of the *E. coli* isolates indicated group A (particularly A_1) was the predominant phylogenetic group (19/34, 55.9%), followed by groups B1 (9/34, 26.5%) and D (6/34, 17.6%); none of the isolates belonged to group B2. The study shows that ESBL-producing *E. coli* isolates exist in the intestinal flora of healthy cattle and sheep in the Burdur province of Turkey. This is the first report showing the emergence of CTX-M type ESBL-producing *E. coli* in sheep farms in Turkey.

Key words: cattle, ESBL, *Escherichia coli*, multidrug-resistance, sheep

INTRODUCTION

Extended-spectrum beta-lactamases (ESBLs) are hydrolytic enzymes produced by Gram-negative bacteria, and they confer resistance to many important antibiotics including penicillins, 1st - 4th generation cephalosporins and monobactams; ESBLs are not active against carbapenems (e.g., imipenem, meropenem and ertapenem) or cephamycins (e.g., cefoxitin). ESBLs are usually inhibited by beta-lactamase inhibitors (e.g., clavulanic acid and tazobactam), which are commonly utilized for laboratory

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detection and confirmation of ESBLs [1-3]. In the recent years, there has been a steady increase in the emergence of ESBL-producing members of *Enterobacteriaceae* around the globe, which presents a major challenge for healthcare and is in part a consequence of selective pressure generated by the extensive use of oxyimino-cephalosporins in the treatment of bacterial infections [4]. The most frequently encountered ESBLs in *Enterobacteriaceae* belong to the TEM, SHV and CTX-M families [1,3]. TEM and SHV variants with ESBL activity have been largely derived from TEM-1/TEM-2 and SHV-1 beta-lactamases respectively [5]. On the other hand, $bla_{\text{CTX-M}}$ genes have been captured from the chromosome of *Kluyvera* spp. onto the conjugative plasmids that mediate their dissemination among *Enterobacteriaceae* [6]. CTX-M enzymes can be subclassified into clusters 1, 2, 8, 9 and 25, based on similarities in amino acid sequences [7].

The presence of ESBL-producing *Escherichia coli* has been described in cattle and sheep populations around the world [8-12]. However, very limited information is available on the presence and extent of ESBL-producing bacteria in cattle and sheep populations in Turkey. To date, only a few local studies [13-15] have been conducted, and the majority focused only on the phenotypic detection of ESBL-producing *E. coli*, without detailed characterization of the ESBL types involved. However, in a small-scale study conducted by Kucukbasmaci et al. [15], ESBLs detected in fecal *Enterobacteriaceae* isolates from cattle and sheep in northwest of Turkey were identified, and none of them were of the CTX-M type. This finding was somewhat surprising considering that CTX-M has been increasingly identified in many different sources including humans, animals and the environment and that it has virtually displaced the other ESBLs within *Enterobacteriaceae* during the last decade [16]. Therefore, the present study was conducted to characterize the ESBL genes found in fecal *E. coli* isolated from healthy cattle and sheep.

MATERIAL AND METHODS

Study population and sampling

The present study was conducted on dairy cattle and sheep populations in Burdur province located in the southwest of Turkey. The study included 16 herds of dairy cattle (Holstein) and 12 flocks of sheep (Awassi) selected using the random sampling method. For sample collection, 200 healthy cattle (≥ 12 months of age) and 200 healthy sheep (≥ 6 months of age) were selected by random sampling. Fecal samples from each cow and sheep were taken directly from the rectum.

Selective isolation and confirmation of ESBL-producing isolates

An enrichment procedure was performed to increase the total bacterial population before culturing the fecal samples for ESBL-producing *E. coli*. A 10% suspension of fecal sample in buffered peptone water (Lab M, UK) was prepared and mixed using a vortex mixer. After incubation of the suspension at 37ºC for 24 hours under

aerobic conditions, 50 µl was evenly spread onto Brilliance *E. coli*/coliform selective agar (Oxoid, UK) supplemented with cefotaxime (CTX, 2 µg/ml) (Sigma-Aldrich, Germany) or ceftazidime (CAZ, 2 µg/ml) (Sigma-Aldrich, Germany) at the same time and incubated for another 24 hours at 37ºC under aerobic conditions.

One colony from each plate (one colony from the selective agar supplemented with CTX and one from the selective agar supplemented with CAZ) per positive sample was selected randomly and subcultured on Tryptic Soy agar (Oxoid, UK) for identification. After *E. coli* identification using conventional methods (Gram staining, acid and gas from glucose, catalase test, citrate utilization, decarboxylation of lysine, hydrogen sulphide production, indole production, methyl red-voges proskauer test, orthonitrophenyl-beta-D-galactopyranoside activity, oxidase test and urease production) [17], the isolates were subjected to genetic confirmation by PCR amplification of a 401 bp fragment of the *E.coli* 16S rRNA gene [18].

ESBL production by *E. coli* isolates was confirmed using the combined disc method recommended by the Clinical and Laboratory Standards Institute (CLSI)[19].

Antibiotic susceptibility testing

One isolate from each medium supplemented with CTX or CAZ per positive sample was subjected to susceptibility testing against nine beta-lactam antibiotics using the agar disc diffusion test following CLSI protocols [19].The tested antibiotic discs (Oxoid, UK) that were: ampicillin (AMP 10 µg), aztreonam (ATM 30 µg), cefepime (FEP 30 µg), cefoxitin (FOX 30 µg), cefpodoxime (CPD 10 µg), ceftriaxone (CRO 30 µg), cefuroxime (CXM, 30 µg), cephalothin (CEF 30 µg), and imipenem (IPM 10 µg). Results were evaluated in accordance with CLSI criteria [19, 21].

In addition to susceptibility to beta-lactam antibiotics, the isolates were also tested for susceptibility to aminoglycosides (gentamicin: GEN, kanamycin: KAN, streptomycin: STR), quinolones (ciprofloxacin: CIP, enrofloxacin: ENR and nalidixic acid: NAL), folate pathway inhibitors (sulfamethoxazole-trimethoprim: SXT), phenicols (florfenicol: FFC) and tetracyclines (tetracycline: TET) using the agar disc diffusion test recommended by CLSI [19]. The antibiotic discs (Oxoid) that were tested were: CIP (5 µg), ENR (5 µg), FFC (30 µg), GEN (10 µg), KAN (30 µg), NAL (30 µg), STR (10 µg), SXT (23.75 + 1.25 µg) and TET (30 µg). Results were evaluated using CLSI criteria [19-21].

The isolates were classified as resistant, intermediate or susceptible [19-21]. *E. coli* isolates of a single fecal sample cultured on the two selective media containing CTX or CAZ and with the same antibiotic susceptibility profile were considered to be the same isolate in this study. Multidrug-resistance was defined as resistance to at least 3 different classes of antibiotics excluding beta-lactams.

Polymerase chain reaction and sequencing

DNA from *E. coli* isolates with confirmed ESBL production was extracted using a genomic DNA purification kit (Thermo Fisher Scientific Inc., Massachusetts, USA) and tested by PCR with specific primers for the bla_{TEM}, bla_{SHV} and bla_{CTX-M} genes as described elsewhere [22-27] with slight modifications in cycling conditions. *Tag* DNA polymerase enzyme, deoxyribonucleotide triphosphates and buffers used in the PCR mixture were obtained from Thermo Fisher Scientific Inc. (Massachusetts, USA). The cycling conditions for detection of the bla_{TEM} gene were as follows: initial denaturation at 94 ºC for 5 min, 35 cycles of 94 ºC for 1 min, 48 ºC for 1 min and 72 ºC for 1 min, with a final elongation at 72 ºC for 10 min. The cycling conditions for bla_{SHV} gene detection were initial denaturation at 94 ºC for 5 min, 35 cycles of 94 ºC for 30 sec, 58 ºC for 30 sec and 72 ºC for 1 min, with a final elongation at 72 ºC for 7 min. The cycling conditions for bla_{CTX-M} gene (universal) detection were initial denaturation at 94 ºC for 5 min, 35 cycles of 94 ºC for 30 sec, 54 ºC for 30 sec and 72 ºC for 1 min, with a final elongation at 72 ºC for 7 min. The cycling conditions for detection of bla_{CTX-M} group 1, 2, 8/25 and 9 genes were as follows: initial denaturation at 94 ºC for 5 min, 35 cycles of 94 ºC for 1 min, 55 ºC for 1 min and 72 ºC for 1 min, with a final elongation at 72 ºC for 7 min.

E. coli ATCC 35218 (bla_{TEM-1}) and *K. pneumoniae* ATCC 700603 (bla_{SHV-18}) were used as positive control strains for bla_{TEM} and bla_{SHV} encoding genes. *E. coli* NCTC 13461, *E. coli* NCTC 13462, *E. coli* NCTC 13463, *Enterobacter cloacae* NCTC 13464 and *K. pneumoniae* NCTC 13465 were used as positive controls for the detection of bla_{CTX-M} group 1, group 2, group 8, group 9 and group 25 genes, respectively. *E. coli* ATCC 25922 was used as a negative control for all PCRs.

To demonstrate bla_{CTX-M} and bla_{TEM} gene diversity in the cattle population, sequence analysis of the respective genes was performed. *E. coli* isolates were selected according to their antibiotic susceptibility profiles and phylogenetic groups. Nine *E. coli* isolates belonging to three phylogenetic groups (A, B1 and D) with nine different antibiotic susceptibility profiles were selected from all of the cattle farms positive for ESBL (n= 8) for further study. For sequence analysis of the bla_{TEM} gene, 10 *E. coli* isolates (from five farms) belonging to three phylogenetic groups (A, B1 and D) with nine different antibiotic susceptibility profiles were also included in the study. To determine bla_{SHV} gene diversity, we sequenced all of the PCR products (n= 3) that were obtained even if the *E. coli* isolates were from a single farm and belonged to the same phylogenetic group. All PCR products (3 bla_{CTX-M} and 2 bla_{TEM}) from *E. coli* isolates from sheep were sequenced. DNA sequencing of PCR products was performed by Refgen Genetical Research and Biotechnology (Golbasi-Ankara, Turkey). Sequencing was carried out on both strands using the same primer pairs that were used in the PCR. These sequences were then compared to the NCBI GenBank sequences using BLAST to confirm the subtypes of beta-lactamase genes. Finally, these sequences were submitted to the NCBI GenBank.

Phylogenetic analysis

To reveal whether ESBL-producing *E. coli* isolates belonging to a particular phylogenetic group were more likely to carry ESBL genes, phylogenetic typing (A, B1, B2 and D) of the isolates was performed according to a triplex PCR protocol as described [28] with modified PCR conditions [29]. To enhance strain discrimination, subgroups (A: A_0 and A_1; B2: $B2_2$ and $B2_3$; D: D_1 and D_2) were also identified as previously described [30].

RESULTS

Detection of ESBL-producing E. coli from cattle and sheep feces

E. coli grew on both types of selective media (supplemented with CTX or CAZ) in 47 of the fecal samples (45 cattle and 2 sheep). The number of isolates grown on only medium containing CTX was five cattle and one sheep isolate but with only medium containing CAZ, there was only one cattle isolate. Overall, presumptive ESBL-producing *E. coli* were isolated from the fecal samples of 51 cattle and 3 sheep. Further characterization using the combined disk method confirmed that 31 of the 51 *E. coli* cattle isolates and all of the *E. coli* sheep isolates produced ESBL. Therefore, 15.5% (31/200) and 1.5% (3/200) of cattle and sheep fecal samples, respectively, were positive for ESBL-producing *E. coli*. Of the farms tested in this study, ESBL-producing *E. coli* was obtained from 50% (8/16) of the cattle herds and 25% (3/12) of the flocks of sheep.

Antimicrobial susceptibility of ESBL-producing E. coli strains

In antibiotic susceptibility testing for the nine beta-lactams, high resistance rates were detected in the ESBL-producing *E. coli* isolates from both cattle and sheep (Table 1). The resistance rates in the cattle isolates against ATM, CPD, CTX, CAZ and CRO, which are used in CLSI initial screening test for ESBL-producing *E. coli*, were 100%, 96.8%, 100%, 80.6% and 96.8%, respectively (Table 1).

Among the *E. coli* cattle isolates confirmed as ESBL-producing, the highest resistance rate against aminoglycosides was found for STR (71.0 %, 22/31). NAL resistance was found in 38.7% of the isolates (12/31), followed by ENR (35.5%, 11/31) and CIP (29.0%, 9/31). In addition, 48.4% (15/31) and 93.5% (29/31) of the cattle isolates were resistant to SXT and TET, respectively (Table 1). Among the ESBL-producing *E. coli* isolates from sheep, resistance was found against GEN only (66.7%, 2/3). None of the sheep isolates showed resistance against CIP, ENR, NAL, TET, SXT and FFC (Table 1). While 45.2% (14/31) of cattle isolates showed multidrug-resistance phenotypes, none of the sheep isolates were multidrug-resistant.

Table 1. Susceptibility of ESBL-producing fecal *E. coli* isolates (n = 34) from cattle and sheep against beta-lactams and other classes of antibiotics

Beta- lactams	Cattle (n = 31)		Sheep (n = 3)		Other antibiotics	Cattle (n = 31)		Sheep (n = 3)	
	R (n)	I (n)	R (n)	I (n)		R (n)	I (n)	R (n)	I (n)
AMP	31	0	3	0	GEN	11	3	2	0
ATM	31	n/a	3	n/a	KAN	14	10	0	2
FEP	7	10	3	0	STR	22	9	0	2
CTX	31	n/a	3	n/a	CIP	9	1	0	0
FOX	0	0	0	0	ENR	11	5	0	0
CPD	30	n/a	3	n/a	NAL	12	3	0	0
CAZ	25	n/a	3	n/a	FFC	2	8	0	0
CRO	30	n/a	3	n/a	SXT	15	1	0	0
CXM	31	0	3	0	TET	29	1	0	0
CEF	31	0	3	0					
IPM	0	0	0	0					

R, resistant; I, intermediate; n/a, not applicable.

Molecular characterization of ESBL types

PCR screening for the bla_{TEM}, bla_{SHV} and bla_{CTX-M} genes in phenotypically-confirmed ESBL-producing *E. coli* isolates of cattle origin indicated that CTX-M was the most common ESBL type, detected in 87.1% (27/31) of the isolates, followed by TEM (77.4%, 24/31) and SHV (9.7%, 3/31). In the ESBL-producing *E. coli* isolates from sheep, bla_{CTX-M} (100%, 3/3) and bla_{TEM} genes (66.7%, 2/3) were detected, but none of the isolates carried bla_{SHV} genes. Group-specific PCR indicated that all of the bla_{CTX-M} genes detected in *E. coli* isolates of both cattle and sheep belonged to CTX-M group 1.

Multiple beta-lactamase genes were detected in the majority of the *E. coli* isolates tested in the study. It was determined that 67.7% (21/31) of the isolates from cattle and 66.7% (2/3) of the isolates from sheep were carriers of both bla_{TEM} and bla_{CTX-M} genes. Each of the bla_{CTX-M} + bla_{SHV} and bla_{TEM} + bla_{SHV} gene combinations were found in a single cattle isolate while the sheep isolates did not carry these gene combinations. None of the isolates tested in the study included the bla_{CTX-M}, bla_{TEM} and bla_{SHV} genes together.

Of the bla_{CTX-M} genes detected in the 27 *E. coli* isolates from cattle, nine were selected for further DNA sequencing. One isolate was CTX-M-3, two isolates were CTX-M-1 and six isolates were CTX-M-15 type ESBL-producers (Table 2). All PCR products of bla_{CTX-M} genes (n = 3) from the sheep isolates were also sequenced; one isolate was CTX-M-3 and two isolates were CTX-M-15 producers (Table 2). Since all three of

these CTX-M types belong to the CTX-M-1 cluster, this finding indicates agreement between sequencing and group-specific PCR. Among the 24 bla_{TEM} genes detected in cattle isolates, 10 were also selected for sequence analysis, and all were found to encode TEM-1 type beta-lactamase (Table 2). Furthermore, sequence analysis of two bla_{TEM} genes detected from sheep isolates confirmed to have the TEM-1 genotype (Table 2). Sequencing of the three bla_{SHV} genes from the cattle isolates indicated the presence SHV-12 type ESBL (Table 2).

Table 2. Distribution of ESBL types of fecal *E. coli* isolates from cattle and sheep according to animal farms and phylogenetic groups

Farm	Number of isolates	Phylogenic group	ESBL Type
Cattle			
A	3	D (subgroup D_2)	CTX-M-1 (n = 1); CTX-M group 1[a](n = 2)
B	1	D (subgroup D_1)	CTX-M-1
C	1	D (subgroup D_1)	CTX-M-15
D	12		
		A (subgroup A_1) (n = 8)	TEM-1 (n = 1); CTX-M group 1[a]+ TEM[a] (n = 5); CTX-M group 1[a]+ TEM-1 (n = 1); CTX-M-3 + TEM[a] (n = 1)
		B1 (n = 3)	SHV-12 (n = 1); SHV-12 + TEM[a] (n = 1); SHV-12 + CTX-M group 1[a] (n = 1)
		D (subgroup D_1) (n = 1)	TEM-1
E	3	A (subgroup A_1)	CTX-M-15 + TEM-1 (n = 1); CTX-M group 1[a] + TEM[a] (n = 2)
F	2	B1	CTX-M-15 + TEM-1 (n = 1); CTX-M group 1[a] + TEM[a] (n = 1)
G	2	B1	CTX-M group1[a] + TEM-1 (n = 1); CTX-M-15 + TEM[a] (n = 1)
H	7		
		A (subgroup A_0) (n = 2)	CTX-M group 1[a] + TEM-1 (n = 2)
		A (subgroup A_1) (n = 4)	CTX-M group 1[a] + TEM[a] (n = 1); CTX-M-15 + TEM[a] (n = 2); CTX-M group 1[a] + TEM-1 (n = 1)
		B1 (n = 1)	CTX-M group 1[a] + TEM-1
Sheep			
A	1	A (subgroup A_1)	CTX-M-15 + TEM-1
B	1	B1	CTX-M-15 + TEM-1
C	1	A (subgroup A_1)	CTX-M-3

[a]not sequenced

Assigned accession numbers for $bla_{CTX-M-1}$, $bla_{CTX-M-3}$, $bla_{CTX-M-15}$ and bla_{SHV-12} gene nucleotide sequence data submitted in GenBank are as follows. $bla_{CTX-M-1}$: F11 (KP162338) and F23 (KP162339). $bla_{CTX-M-3}$: F62 (KP303590) and F187 (KP303592). $bla_{CTX-M-15}$: F50 (KP325140), F54 (KP325141), F85 (KP325142), F97 (KP325143),

F128 (KP325144), F130 (KP325145), F147 (KP325146) and F170 (KP325147). bla_{SHV-12}: F57 (KP100155), F58 (KP100154) and F68 (KP162337).

Phylogenetic types of ESBL-producing E. coli strains

Of the 31 *E. coli* isolates of cattle origin that were analyzed, 17 (54.8%) belonged to phylogenetic group A, eight (25.8%) to group B1, and six (19.4%) to group D. Most of the group A isolates of cattle origin (15/17, 88.2%) belonged to subgroup A_1. Of the three *E. coli* isolates of sheep origin, two (66.7%) were in group A (subgroup A_1) and the third strain (33.3%) was in group B1. None of the cattle and sheep isolates belonged to group B2, the phylogenetic group most likely to be highly virulent. Distribution of the isolates according to phylogenetic groups along with the included ESBL types is given in Table 2.

Of the eight cattle herds which were positive for ESBL-producing *E. coli*, six farms had more than one isolate. Three isolates which belonged to phylogenetic group D (subgroup D_2) and three isolates which belonged to group A (subgroup A_1) were identified on farm A and E, respectively. On farms F and G, two isolates from phylogenetic group B1 were identified. Nevertheless on farm D, 12 isolates from three different phylogenetic groups were detected and they belonged to group A (subgroup A_1, n = 8), group B1 (n = 3) and group D (subgroup D_1, n = 1). On farm H, seven isolates were distributed in two different phylogenetic groups, A (subgroup A_0, n = 2; and subgroup A_1, n = 4) and B1 (n = 1). The SHV-12 type ESBL-producing *E. coli* isolates were from Farm D, and all isolates belonged to phylogenetic group B1. However, the additional beta-lactamase genes they carried were different; one strain carried only the bla_{SHV-12} gene, the second carried both the bla_{SHV-12} and bla_{TEM} gene and the third had the bla_{SHV-12} and bla_{CTX-M} gene (Table 2).

DISCUSSION

Emergence and dissemination of ESBL-producing *Enterobactericeae* of animal and human origin is increasing, which is a cause for considerable concern to both medical and veterinary practitioners around the world. A number of investigations have been conducted in various parts of the world to investigate the presence and types of ESBL in cattle [8-12, 31-33], but research on ESBL in sheep is limited [8,11,33]. In Turkey, only one study has been conducted so far in which both the presence and types of ESBLs in cattle and sheep were investigated, and this was in the northwest of Turkey [15]. That study reportedly identified only three ESBL-producing *E. coli* isolates in cattle and none in sheep. Therefore, our study represents the first report of the presence of ESBL-producing *E. coli* isolates from sheep in Turkey.

The increase in the prevalence of ESBL-producing *E. coli* may be due to the clonal spread of certain ESBL-producing strains and/or horizontal transfer of ESBL-plasmids between strains of different genomic background [5]. Although the types of

ESBLs produced by *E. coli* differ depending on the animal population and geographical areas, detection rates of CTX-M type ESBLs have increased dramatically around the world during the last several years [1, 16, 34]. In line with this trend, the present study found that the bla_{CTX-M} gene was the most common ESBL type detected in the phenotypically confirmed ESBL-producing *E. coli* isolates.

Among CTX-M type ESBLs, CTX-M-1, CTX-M-14 and CTX-M-15 are the most widespread and predominant ones detected in many studies reported from various countries [33,35-37]. In bovine *E. coli* strains, CTX-M-1, -14, and -15 types in France [36,37], CTX-M-14 and -15 types in the UK [35], and CTX-M-14 and -15 types in Wales [33] have been reported. In sheep, *E. coli* strains producing CTX-M-1, -14 and -15 types were detected in Switzerland [11]. Similar to the findings of these studies, CTX-M-15 was also found to be the most common ESBL-CTX-M type detected in fecal *E. coli* isolates from cattle and sheep in our study.

DNA sequencing of the bla_{TEM} genes identified in *E. coli* isolates from cattle and sheep has shown that all of the isolates are TEM-1 type, which is not considered an ESBL [1]. However, of the 10 *E. coli* isolates of cattle origin carrying bla_{TEM-1}, nine also carried the bla_{CTX-M} gene and all of the *E. coli* isolates (n= 2) of sheep origin with bla_{TEM-1} also carried the bla_{CTX-M} gene. Only two cattle strains had bla_{TEM-1} alone, yet exhibited the ESBL phenotype. This is likely due to the production of other ESBL types that were not investigated in the present study.

Intensive use of beta-lactams and other classes of antibiotics in the livestock industry may have contributed to the emergence of multidrug-resistant bacterial phenotypes. In Turkey, beta-lactams, aminoglycosides, phenicols, quinolones, folate pathway inhibitors and tetracycline**s** are widely used in cattle and sheep production for the treatment of a variety of infections (for example, enteritis, mastitis, pneumonia and septicemia). Studies performed in Turkey show that *E. coli* isolates of cattle origin are generally more resistant to various antibiotics than isolates of sheep origin [13, 38]. Likewise, we found that the overall antibiotic resistance rates of other classes in the ESBL-producing *E. coli* isolates of cattle origin were higher than those of the sheep isolates. While resistance was observed against CIP, ENR, NAL, FFC, SXT and TET in the cattle isolates, the sheep isolates were not resistant to these antibiotics. Additionally, multidrug-resistant phenotypes were observed in only *E. coli* isolates of cattle origin in the present study. The higher resistance in the cattle isolates can be attributed to use of these antibiotics more widely in the treatment of a wide variety of infections in the cattle population and co-selection of resistant isolates.

Phylogenetic grouping of *E. coli* strains shows that most commensal strains generally belong to groups A and B1, whereas group B2, and to lesser extent group D, are generally associated with virulent extraintestinal strains [28,39]. In our study, the predominant phylogenetic group was group A (particularly subgroup A_1), followed by group B1 and group D. Even though none of the isolates in our study belonged to the B2 phylogenetic group, which represents the highly virulent extraintestinal *E.*

coli strains, we found six *E. coli* isolates in the group D cluster, meaning that some of the isolates may be also pathogenic. On the other hand, Milanov et al. [40] reported *E. coli* strains from phylogenetic groups A and B1 isolated from bovine mastitis cases, which shows that commensal *E. coli* strains from group A and B1 can cause various infections in cattle.

The presence of ESBL-producing *E. coli* isolates from more than one phylogenetic group indicates that there is significant diversity among *E. coli* isolates carrying ESBL genes in the cattle herds and sheep flocks in this region. This is especially supported by the presence of *E. coli* isolates from three different phylogenetic groups on cattle farm D and two different phylogenetic groups on cattle farm H.

In conclusion, our study shows that $bla_{\text{CTX-M}}$ group 1 ESBL genes (especially $bla_{\text{CTX-M-15}}$) are predominant in commensal *E. coli* isolates in cattle and sheep in Burdur province. This is the first report of the presence of this gene in *E. coli* isolated from sheep in Turkey. However, additional studies using a broader population should be conducted in order to better understand the epidemiology of ESBL genes in animals in Turkey. Furthermore, the veterinary practitioners and farmers should be informed of this important problem and encouraged to be prudent in the use of antimicrobials for animals.

Acknowledgements

The present study was funded by The Scientific and Technological Research Council of Turkey (TUBITAK project number, 112O 820) and partially by Mehmet Akif Ersoy University, Scientific Research Projects Unit (Turkey) (Project number, 0158 KAYDEP-13). We thank biologist Mert Sudagidan (Scientific and Technological Application and Research Center, Mehmet Akif Ersoy University, Burdur, Turkey) for technical assistance.

Authors' contributions

FP designed the study, and carried out the sample collection, isolation and identification of the bacterial isolates and molecular experiments (PCR and DNA sequencing), and drafted the manuscript. HT participated in the design of the study and the laboratory experiments and helped to draft the manuscript. DO participated in the sample collection and the laboratory experiments, and helped to draft the manuscript. HY participated in the design of the study and helped to draft the manuscript. All authors read and approved the final manuscript.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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MOLEKULARNA KARAKTERIZACIJA ESBL-PRODUKUJUĆIH *ESCHERICHIA COLI* IZOLATA IZ ZDRAVIH GOVEDA I OVACA

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Cilj studije je bio karakterizacija *Escherichia coli* izolata koji proizvode ESBL, a koji su izolovani iz zdravih goveda i ovaca u Burdur proviniciji u Turksoj. Uzorci fecesa od ukupno 200 goveda i 200 ovaca su ispitivani pri čemu je 31 uzorak od goveda (15,5%) i 3 uzoraka od ovaca (1,5%), bilo pozitivno na ESBL-produkujuće *E. coli*. Upotrebljen je kombinovani klinički i laboratorijski standardni metod Instituta. Od svih ESBL klasa

gena, koji su ustanovljeni PCR metodom, bla_{CTX-M} tip je bio najčešći, a sledile su bla_{TEM} i bla_{SHV} familije. Izolati *E. coli* koji proizvode ESBL pokazali su ko-rezistanciju na veći broj različitih antibiotika uključujući aminoglikozide, fenikole, quinolone, inhibitore folatnog puta i tetracikline. Stepen rezistencije je bio veći kod izolata poreklom od goveda, u poređenju sa izolatima od ovaca. Filogenetsko grupisanje *E. coli* izolata ukazuje da je grupa A (naročito A1) bila dominantna (19/34, 55,9%), pri čemu su sledile grupe B1 (9/34, 26,5%) i D (6/34, 17,6%); nijedan izolat nije spadao u B2 grupu. Studija pokazuje i to da se ESBL-produkujući *E. coli* izolati, nalaze u intestinalnoj flori zdravih goveda i ovaca u Burdur pokrajini Turske. Ovo je prvi prikaz koji ukazuje na pojavu CTX-M tipa ESBL-produkujućih E. coli iziolata iz ovaca na farmama u Turskoj.