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Research Article

Prevalence and Characterization of ESBL- and AmpC-producing *Escherichia coli* from Cattle [1]

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Abstract

In this study, it was aimed to determine the prevalence of extended spectrum β-lactamase (ESBL) and/or AmpC type β-lactamase (AmpC) producing *Escherichia coli* from cattle in Hatay. For this purpose, 312 rectal swabs samples were collected from apparently healthy cattle. ESBL production was phenotypically investigated by disc combination method and double disc synergism test and β-lactamase genes (bla_{CTX-M}, bla_{CMY-2}, bla_{SHV}, bla_{OXA}, *and* bla_{TEM}) and plasmid mediated quinolone resistance (PMQR) genes (*qnr*A, *qnr*B, *qnr*S and *aac*(6')-Ib) were screened by polymerase chain reaction (PCR) and subsequent sequence analysis. Antimicrobial susceptibility of the isolates were determined using disc diffusion method and their phylogenetic groups were also searched by PCR. Twenty six (8.3%) isolates were found to be ESBL producer by phenotypic tests. The following ESBL/AmpC genes were detected: $bla_{CTX-M-15}$ (n= 12), $bla_{CTX-M-1}$ (n=11), $bla_{CTX-M-3}$ (n=2), and bla_{CMY-2} (n=1). PMQR genes were detected in 11 (42.3%) ESBL producing *E. coli* isolates and these isolates were only positive for *aac(6')-Ib-cr* and *qnr*S1 genes. Twenty two (84.6%) of the isolates exhibited multidrug resistance (MDR) phenotype. ESBL/AmpC producing *E. coli* isolates were observed to be belonged to B1 (50%), A (34.6%) and D (15.4%) phylogroups. This study was the first to describe the presence of CTX-M-15, CTX-M-3, CTX-M-1 and CMY-2 producing *E. coli* in cattle in Turkey and the co-existence of *aac(6')-Ib-cr* and *qnr*S1 genes in some isolates.

Keywords: *Escherichia coli, Cattle, ESBL, AmpC*

Sığırlarda GSBL ve AmpC Sentezleyen *Escherichia coli*'nin Prevalansı ve Karakterizasyonu

Özet

Bu çalışmada, Hatay ilinde sığırlarda genişlemiş spektrumlu β-laktamaz (GSBL) ve/veya plazmid aracılı AmpC tip β-laktamaz (AmpC) sentezleyen *E. coli*'nin prevalansının belirlenmesi hedeflendi. Bu amaçla, sağlıklı görünüşlü 312 sığırdan rektal sıvab örneği toplandı. Fenotipik GSBL sentezi disk kombinasyon metodu ve çift disk sinerji testi ile β-laktamaz (bla_{CTX-M}, bla_{CMY-2}, bla_{SHV}, bla_{OXA} ve bla_{TEM}) ve plazmid aracılı kinolon direnç (PAKD) genleri (*qnr*A, *qnr*B, *qnr*S ve *aac*(6')-Ib) polimeraz zincir reaksiyonu (PZR) ve daha sonra sekans analizi ile araştırıldı. Ayrıca, GSBL ve/veya AmpC sentezleyen *E. coli* izolatlarının antimikrobiyal duyarlılıkları disk difüzyon metodu ile filogenetik gruplarının belirlenmesi ise PZR ile incelendi. Yirmialtı izolat (%8.3) GSBL üretimi yönünden fenotipik testlerle pozitif bulundu. Bu izolatlarda $bla_{CTX-M-15}$ (n= 12), $bla_{CTX-M-1}$ (n= 11), $bla_{CTX-M-3}$ (n= 2) ve bla_{CMY-2} (n=1) genleri belirlendi. PAKD genleri 11 (%42.3) ESBL sentezleyen *E. coli* izolatında saptandı ve bu izolatlar sadece *aac(6')-Ib-cr* ve *qnr*S1 genleri yönünden pozitif bulundu. İzolatlarının 22'si (%84.6) çoğul direnç fenotipi gösterdi. GSBL/AmpC sentezleyen *E. coli* izolatlarının B1 (%50), A (%34.6) ve D (%15.4) filogruplarına ait olduğu görüldü. Bu çalışma ile, ilk kez Türkiye'de sığırlarda CTX-M-15, CTX-M-3, CTX-M-1 ve CMY-2 tip β-laktamaz sentezleyen *E. coli* varlığı ve bu izolatların bazılarında *aac(6')-Ib-cr* ve *qnr*S1 genlerinin birlikte bulunduğu gösterilmiştir.

Anahtar sözcükler: *Escherichia coli, Sığır, GSBL, AmpC*

INTRODUCTION

Emergence and dissemination of extended spectrum β-lactamase (ESBL) and/or AmpC type β-lactamase (AmpC) producing *Escherichia coli* are public health concern worldwide in the intestinal microbiota of food-producing animals [1]. Since ESBL and/or AmpC producing *E. coli* isolates frequently contain resistance genes to other classes

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of antimicrobial (e.g. fluoroquinolones, aminoglycosides and tetracyclines), therapeutic options are very limited [2]. Another public concern is the transmission of these bacteria to humans through the food chain [1]. Even though different ESBL types have been reported in *E. coli* isolates from food-producing animals, CTX-M have widely been encountered not only in humans but also in animals [3].

AmpC type β-lactamases are either chromosomally mediated or plasmid-mediated in the *Enterobactericeae*. In contrast to ESBLs, they hydrolyze cephamycins (cefoxitin and cefotetan) and are not inhibited by β-lactamase inhibitors. Therefore, cefoxitin insusceptibility has been used for screening of AmpC producers. Despite the fact that AmpC type β-lactamases are of numerous types, CMY-2 is the most encountered in Enterobactericeae members from different host [4]. So far, the presence of AmpC producing *E. coli* has not yet been shown in food producing animals in Turkey.

Data on the occurrence and dissemination of ESBL/AmpC producing *E. coli* in cattle are very scarce in Turkey [5]. Therefore, the objectives of this study were to determine the prevalence of ESBL and/or AmpC producing *E. coli* from cattle. Antimicrobial resistance profiles and the phylogenetic groups of the isolates were also studied.

MATERIAL and METHODS

Bacterial Isolates

Rectal swabs (n=312), of which 172 were taken from dairy cattle and 140 from beef cattle, were collected from different cattle farms located in Hatay, Turkey, from March 2012 to June 2013. The study was approved by the Animal Ethical Committee of Mustafa Kemal University (2012/95). The rectal swabs were streaked onto Eosin Methylen Blue (EMB) agar supplemented with cefotaxime (2 µg/mL) and incubated at 35°C for 24 h. One typical colony for *E.coli* per plate was selected and identified by conventional methods and confirmed by polymerase chain reaction (PCR) [6].

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility of the isolates for confirmed ESBL producer *E. coli* isolates were determined by disk diffusion method in accordance with Clinical Laboratory Standards Institute guidelines (CLSI) [7]. The following antimicrobial disks (Bioanalyse, Turkey) were used: ampicillin (10 µg), amoxicillin/clavulanic acid (20 µg/10 µg), cefotaxime (30 µg), ceftazidime (30 µg), cefoxitin (30 µg), imipenem (10 µg), gentamicin (10 µg), streptomycin (10 µg), kanamycin (30 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), sulfamethoxazole-trimethoprim (1.25 µg/23.75 µg), tetracycline (30 µg) and chloramphenicol (30 µg). The isolates resistant to third generation cephalosporins were confirmed as ESBL producer by double disk synergy [8] and disk combination method according to guidelines of CLSI [7]. Cefoxitin resistance were considered positive for AmpC production [7]. All phenotypically positive isolates were examined for ESBL/AmpC genes. *E. coli* standard strain ATCC 25922 were used for quality control.

Phylogenetic Grouping

The ESBL/AmpC producing *E. coli* isolates were phylogenetically grouped into A, B1, B2 or D using the triplex PCR reaction as previously reported by Clermont et al.[9].

Detection of ESBL, AmpC and PMQR Genes

ESBL genes (bla_{CTX-M}, bla_{TEM}, bla_{SHV} and bla_{OXA}) [10], bla_{CMY-2} [11], and plasmid-mediated quinolone resistance (PMQR) genes (*qnr*A, *qnr*B, *qnr*S and *aac(6')-Ib*) [12,13] were investigated as previously reported.

Statistical Analysis

Statistical differences between beef and dairy cattle were investigated by use of IBM SPSS Statistics package Version 23 (IBM Corp., Armonk, NY, USA). Differences were considered significant at $P<0.05$.

RESULTS

Out of 312 rectal swab samples, 26 (8.3%) *E. coli* isolates were ESBL/AmpC phenotype, comprising 10 (5.8%) from dairy cows and 16 (11.4%) from beef cattle (P=0.075). Following PCR amplification and sequencing of ESBL/AmpC producing *E. coli* strains, 12 (46.2%) harbored $bla_{CTX-M-15}$, 11 (42.3%) $bla_{CTX-M-1}$, two (7.7%) $bla_{CTX-M-3}$ and one (3.8%) bla_{CMY-2}. In addition to ESBL/AmpC genes, other β-lactamase genes were detected in 22 isolates (84.6%), of which 21 isolates harbored bla_{TEM-1b} and one isolate harbored bla_{OXA-1} in combination with ESBL/AmpC genes *(Table 1)*.

Most of ESBL/AmpC producing *E. coli* isolates belonged to group B1 (50%) and group A (34.6%), to lesser extent to group D (15.4%). However, group B2 was not found in the isolates.

All isolates were resistant to ampicillin (100%) but susceptible to imipenem. Isolates were also found to be resistant to streptomycin (80.8%), sulfamethoxazole-trimethoprim (76.9%), tetracycline (73.1%), chloramphenicol (53.8%), kanamycin (50%), amoxicillin/clavulanic acid (46.2%), nalidixic acid (46.2%), ciprofloxacin (23.1%) and cefoxitin (3.8%). Multiple resistance phenotype (resistance to three or more antimicrobials) were found in 22 (84.6%) isolates *(Table 1)*. PMQR genes were only detected in 11 ESBL producing *E. coli* isolates. Among the 11 PMQR positive isolates, eight carried *aac(6')-Ib-cr*, two *qnr*S1 and one both *qnr*S1 and *aac(6')-Ib-cr*. All of the isolates tested were negative for the *qnr*A, *qnr*C, *qnr*D and *qep*A *(Table 1)*.

Table 1. *Characteristics of ESBL/AmpC producing E. coli isolates from cattle*
Tablo 1. *Sığırlardan izole edilen ESBL/AmpC sentezleyen E. coli özellikleri*

Isolate ID	Phylogenetic Group	ESBL Type	Other Beta-lactamase Genes	AmpC Type	PMQR Gene	Antimicrobial Resisitance Phenotype*
22	A	CTX-M-15	TEM-1b	-	-	AMP, S, CN, TE, SXT, C
37	A	-	TEM-1b	CMY-2	*aac(6')-Ib-cr*	AMP, AMC, FOX, S, TE, C
38	D	CTX-M-15	TEM-1b	-	*qnr*S1	AMP, S, TE, SXT, C
40	D	CTX-M-15	TEM-1b	-	-	AMP, S, K, TE, SXT
44	A	CTX-M-15	TEM-1b, OXA-1	-	*aac(6')-Ib-cr*	AMP, AMC, NA, CIP, S, CN, K, TE, SXT
57	B1	CTX-M-15	-	-	*qnr*S1	AMP
60	D	CTX-M-15	TEM-1b	-	-	AMP, NA, S, K, TE, SXT
76	B1	CTX-M-3	-	-	*aac(6')-Ib-cr*	AMP
78	A	CTX-M-15	TEM-1b	-	-	AMP, AMC, S, SXT
80	B1	CTX-M-3	-	-	-	AMP
88	D	CTX-M-15	-	-	-	AMP
192	B1	CTX-M-1	TEM-1b	-	-	AMP, S, TE, SXT
197	B1	CTX-M-1	TEM-1b	-	*aac(6')-Ib-cr*, *qnr*S1	AMP, S, TE, SXT
221	B1	CTX-M-15	-	-	-	AMP, S, CN, TE, SXT, C
224	A	CTX-M-15	TEM-1b	-	-	AMP, S, TE, SXT, C
242	A	CTX-M-15	TEM-1b	-	*aac(6')-Ib-cr*	AMP, AMC, NA, CIP, S, SXT, C
246	A	CTX-M-1	TEM-1b	-	*aac(6')-Ib-cr*	AMP, AMC, NA, CIP, S, K, TE, SXT, C
257	B1	CTX-M-15	TEM-1b	-	*aac(6')-Ib-cr*	AMP, S, K, TE, SXT
276	A	CTX-M-1	TEM-1b	-	-	AMP, AMC, NA, S, K, TE, SXT, C
277	B1	CTX-M-1	TEM-1b	-	-	AMP, NA, S, K, TE, SXT, C
279	A	CTX-M-1	TEM-1b	-	-	AMP, AMC, NA, S, K, TE, SXT, C
280	B1	CTX-M-1	TEM-1b	-	-	AMP, AMC, NA, S, K, TE, SXT, C
282	B1	CTX-M-1	TEM-1b	-	-	AMP, AMC, NA, S, K, TE, SXT, C
283	B1	CTX-M-1	TEM-1b	-	-	AMP, AMC, NA, CIP, S, K, TE, SXT, C
286	B1	CTX-M-1	TEM-1b	-	*aac(6')-Ib-cr*	AMP, AMC, NA, CIP, K
287	B1	CTX-M-1	TEM-1b	-	*aac(6')-Ib-cr*	AMP, AMC, NA, CIP, S, K, TE, SXT, C

****AMP:** ampicillin, **AMC:** amoxicillin/clavulanic acid, **FOX:** cefoxitin, **CN:** gentamicin, **S:** streptomycin, **K:** kanamycin, **NA:** nalidixic acid, **CIP:** ciprofloxacin, **SXT:** sulfamethoxazole-trimethoprim, **TE:** tetracycline; **C:** chloramphenicol*

DISCUSSION

ESBL and/or AmpC producing Enterobacteriaceae have been a growing problem throughout the world [1], and it has increasingly been reported in food producing animals (EFSA) [14]. The prevalence rate of ESBL/AmpC producing *E. coli* in cattle (8.3%) was higher than those previously reported in France (5.8%) [15] and Hong Kong (3.1%) [16], but lower than those reported in Switzerland (16%) [17] and Japan (31.3%) [18]. However, in a study conducted in Poland, no ESBL/AmpC producing *E. coli* were observed [19].

In the current study, beef cattle (11.4%) showed higher prevalence rate than dairy cattle (5.8%) (P=0.075). Ohnishi et al.[20] reported a prevalence rate of 2.6% in dairy farms in Japan, whereas Schmid et al.[21] reported higher prevalence in dairy cattle (41.1%) and in beef cattle (18.9%) in Germany.

Up until now, very little data have been present in literature on the occurence and molecular characterization of ESBL/AmpC producing *E. coli* among food producing animal in Turkey. Küçükbasmacı et al.[5], the first to report ESBL producing *Enterobactericeae* from food producing animals in Turkey, reported a prevalence of 2.1% (5/277) among *Enterobactericeae* from cattle, of which only three isolates were *E. coli*. In their study, the authors detected only OXA-10 and SHV-5 as ESBL enzymes together with TEM-1. Recently, Önen et al.[22] reported higher contamination rate of chicken meat with ESBL producing *E. coli*, but low contamination rate for beef meat (7%) in Turkey. In contrast, Başaran Kahraman et al.[23], who investigated the presence of ESBL and AmpC β-lactamase producing *E. coli* from faecal samples of broiler and egg-type healthy chicken belonging to 43 flocks, reported prevalence of ESBL and AmpC producers as 7.8% and 3.6%, respectively.

Considerable differences in ESBL/AmpC β-lactamase types have been detected in *E. coli* from food producing animals in the world [1]. In this study, CTX-M-15 and CTX-M-1 were the most frequently detected ESBL enzymes, which are also commonly detected in human clinical *E. coli* isolates in Turkey [24,25]. CTX-M-1 was detected as predominant ESBL enzyme in France [15] and Switzerland [17], whereas CTX-M-2 in Japan [20] and CTX-M-14 in China [26] were reported as predominant ESBL enzyme. Moreover, detection of CMY-2 type β-lactamase in one *E. coli* isolate was an important finding that have never been repoted in Turkey so far. The results suggest that there have been a drastic change in the epidemology of ESBL producing *E. coli* in cattle in Turkey.

E. coli strains found on microbioata of farm animals were reported to belong largely B1 and A phylogenetic groups, and to lesser extent, to phylogenetic groups B2 and D [27]. In this study, phylogenetic analysis of *E. coli* strains revealed that ESBL/AmpC producing strains were mainly belonged to group B1 and showed absence of phylogenetic group B2.

In the present study, vast majority of the isolates (84.6%) were also resistant to non-β-lactam antibiotics, and showed multi drug resistance. A similar finding was also reported by Valentin et al.[28], who found that ESBL/AmpC producing *E. coli* strains were also resistant to other class of antimicrobials agents. This could partly be explained by the fact that the plasmids harboring ESBL/AmpC genes frequently carry other resistance genes that are responsible for other class antimicrobials, such as fluoroquinolones, aminoglycosides and trimethoprim-sulphamethoxazole [28].

For the first time, PMQR genes were found together with ESBL/AmpC genes from cattle in Turkey. The most prevalent PMQR gene was a*ac(6')-Ib-cr* (42.3%) whereas *qnr*S1 (11.5%) was the only *qnr* gene detected. This higher co-existence could be explained by overuse and misuse of fluoroquinolones in animals. In accordance with our results, this finding has been previously documented in *E. coli* isolates from animals of different origin [29,30].

In conclusion, the results indicate that cattle are potential reservoir of ESBL/AmpC producing *E. coli* in Turkey. Thus, ESBL/AmpC producing bacteria should be monitored in both microbiota of healthy animals and clinical materials regularly, and prudent use of antimicrobial agents is necessary to prevent spread of these bacteria.

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