Prevalence of β-Lactamase Producing *Escherichia coli* from Retail Meat in Turkey

Sevda Pehlivanlar Önen, Özkan Aslantaş, Ebru Şebnem Yılmaz, and Cemil Kürekci

Abstract: Extended spectrum β-lactamase (ESBL) and plasmid-mediated AmpC β-lactamase (pAmpC) producing *Escherichia coli* have been shown to be present in humans and animals representing a significant problem worldwide. This study aimed to search the presence of ESBL and/or AmpC-producing *E. coli* in retail meats (chicken and beef) in Turkey. A total of 88 β-lactamase-producing *E. coli* were isolated from chicken ($n = 81/100$) and beef meat ($n = 7/100$) samples and their susceptibility to several antimicrobials were tested using disc diffusion method. *E. coli* isolates were further characterized for their phylogenetic groups. β-Lactamase encoding (bla_{TEM}, bla_{SHV}, bla_{OXA}, bla_{CTX-M}, and bla_{AmpC}) and quinolone resistance genes (*qnrA*, *qnrB*, *qnrS*, *qepA*, and *acc(6′)-Ib-cr*) were also secreened by polymerase chain reaction (PCR). However, in regard to β-lactamase genes, 84 of 88 isolates were positive for $bla_{CTX-M-1}$ ($n = 39$), $bla_{CTX-M-3}$ ($n = 5$), $bla_{CTX-M-15}$ ($n = 4$), bla_{TEM-1b} ($n = 2$), bla_{SHV-12} ($n = 1$), $bla_{CTX-M-1}/bla_{TEM-1b}$ ($n = 10$), $bla_{CTX-M-1}/bla_{TEM-1b}/bla_{SHV-5}$ ($n = 1$), $bla_{CTX-M-1}/bla_{CMY-2}$ ($n = 1$) and bla_{TEM-1b}/bla_{CMY-2} ($n = 6$), $bla_{CTX-M-15}/bla_{SHV-12}$ ($n = 1$), $bla_{CTX-M-15}/bla_{TEM-1b}$ ($n = 1$), $bla_{TEM-1b}/bla_{SHV-12}$ ($n = 1$), and bla_{CMY-2} ($n = 12$) genes. Resistance to cefuroxime (75.6% and 85.7%), nalidixic acid (89% and 85.7%), tetracycline (91.4% and 100%), streptomycin (40.2% and 100%), and trimethoprim-sulfamethoxazole (36.6% and 85.7%) was observed among strains isolated from chicken and beef, respectively. However, all isolates were found to be susceptible to amikacin, imipenem, and cefepime. Resistance to ampicillin and cefoxitin was significantly linked to bla_{CMY-2} gene, while there was a significant correlation between CTX-M type ESBL and antimicrobial resistance to cefuroxime and streptomycin ($P < 0.05$). The results of this study suggest that raw chicken retail meats are highly contaminated with ESBL-producing *E. coli* implementing a great risk to human health in Turkey.

Keywords: beef meat, chicken meat, ESBL, *E. coli*

Practical Application: The occurrence of ESBL producing *Escherichia coli* in food of animal origin has been found to be significant risk factors for human health. In order to improve food safety in the chicken industry, it is of importance to determine the carriage of this pathogen and its antimicrobial resistance against critically important antibiotics in human health medicine. In the current study, raw chicken retail meats are highly contaminated with ESBL producing *E. coli* and the situation has to be monitored regularly.

Introduction

Escherichia coli are one of the major causes of urinary tract infections (UTIs) and are associated with gastrointestinal upset, wound infection, neonatal meningitis, pneumonia, and bacteremia (Kaya and others 2013). Treatment of infections caused by *E. coli* is becoming a serious problem because of the presence of resistance against multiple antibiotics including β-lactams (Livermore 2008; Pitout 2010). The presence of β-lactam resistance among *E. coli* appears to be a global increase partly in association with certain antibiotic choices in health care settings (Bradford 2001, Pitout 2010). β-Lactam-resistant *E. coli* have also been frequently found to be resistant to other classes of antimicrobial agents including fluoroquinolones, aminoglycosides, and trimethoprim–sulfamethoxazole (Bradford 2001) resulting increased morbidity and mortality, prolonged hospitalization and increased healthcare costs among hospitalized patients (Perez and others 2007; Pitout 2010; Rupp and Paul 2003).

Plasmid-mediated β-lactamases [extended spectrum β-lactamases (ESBLs) and AmpC β-lactamases (AmpC)] have been of great concern worldwide (Pitout and Laupland 2008). They result in resistance to β-lactam antibiotics (penicillins, cephalosporins, and monobactams) mainly because of the breaking of β-lactam rings. AmpC type β-lactamases are clinically important cephalosporinases that are chromosomally mediated and are also be carried on plasmids in many Gram-negative bacteria (Jacoby 2009; Philippon and others 2002). AmpC β-lactamase, particularly CMY-2, the most widely distributed plasmid-mediated AmpC have been commonly found in Enterobacteriaceae isolated from humans and animals worldwide (Ben Sallem and others 2012). Extended spectrum β-lactameses (ESBLs) usually refers to resistance to the oxyimino-cephalosporins (such as cefotaxime, ceftazidime, and cefepime) (Paterson and Bonomo 2005). There are more than 300 well-described ESBL subtypes globally distributed owing to transmission of many plasmids from cell to cell (Bonnet 2004; Liebana and others 2006) with CTX-M most commonly reported subtype both in humans and animals since the early 2000s (Livermore and others 2007). The rapid increase in the proportion of ESBL-producing *E. coli* in alimentary tract of food producing (poultry, cattle, pigs, and

MS 20150284 Submitted 2/17/2015, Accepted 6/23/2015. *Authors Pehlivanlar Önen and Kürekci are with Dept. of Food Hygiene and Technology, Faculty of Veterinary Medicine, Mustafa Kemal Univ., Hatay, Turkey. Author Aslantaş is with Dept. of Microbiology, Faculty of Veterinary Medicine, Mustafa Kemal Univ., Hatay, Turkey. Author Şebnem Yılmaz is with Dept. of Biology, Faculty of Art and Science, Mustafa Kemal Univ., Hatay, Turkey. Direct inquiries to author Kürekci (E-mail: ckurekci@hotmail.com).*

doi: 10.1111/1750-3841.12984

Journal of Food Science A Publication of the Institute of Food Technologists

sheep) and pet animals have been occurred globally since 2000s (Aarestrup and others 2006; Blanc and others 2006; Brinas and others 2005; Jouini and others 2007). Therefore, prevalence of ESBL-producing *E. coli* has been also appeared to be in increase in various foodstuffs including raw milk, dairy products and meat, particularly chicken meat which has already been recognized as a significant source of ESBL-producing *E. coli* in humans (Overdevest and others 2011). Additionally, plasmids carrying ESBLs genes often contain the genes encoding resistance to other drug classes including quinolone and aminoglycosides. For example, it has been shown that *E. coli* isolates harboring CTX-M type β-lactamases often contain the genes (*qnr*A, *qnr*B or *qnr*S and *aac(6′)-Ib-cr*) conferring quinolone resistance indicating the localization of these genes on the same plasmid (Nordman and Poirel 2005).

To our knowledge, there has been no report on molecular characterization on β-lactam-resistant isolates from foods in Turkey, despite the great importance of ESBLs and/or pAmpC β-lactamase-producing *E. coli* in foods of animal origin. Therefore, the main aim of the present study was to determine the prevalence of β-lactamase-producing *E. coli* in meat samples from retail outlets. The isolates obtained in this study were screened for their resistance to several antimicrobials and further analysed for the presence of ESBLs and AmpC types as well as phylogenetic grouping. In addition, the presence of genes encoding resistance to quinolones (*qnrA*, *qnrB*, *qnrS*, *qepA*, and *acc(6′)-Ib-cr*) were also screened. To our knowledge, this is the first study investigating the genotypes of β-lactamase-producing *E. coli* isolates from raw meat samples in Turkey.

Materials and Methods

Isolation of cefotaxime-resistant *E. coli*

Chicken ($n = 100$) and beef ($n = 100$) samples (frozen or refrigerated) were collected from different markets and butcher shops in Hatay region, Turkey, from February to June in 2012. For the isolation of *E. coli*, each sample (~25 g) was suspended in 225 mL of sterile buffered peptone water and was mixed by stomacher for 1 min. The suspension was incubated at 37 °C for 24 h. At the end of the incubation, 100 μL of the suspension was plated on Eosine Methylene Blue (EMB) agar including cefotaxime (2 μg/mL) and then incubated at 37 °C for 24 h (Leininger and others 2001). One colony showing typical *E. coli* morphology were picked and subcultured on Columbia Blood Agar. The identification was verified by Gram staining and classical biochemical tests (IMVIC). Selected isolates were further confirmed by PCR using species specific primers conserving regions of the 16S rRNA genes (Wang and others 2002). All primers used in this study were listed in Table 1. Isolates were then stored in Tryptic Soy Broth with 20% glycerol at −20 °C.

Phenotypic ESBL tests

ESBL production in cefotaxime-resistant *E. coli* isolates were confirmed by phenotypic disc combination method using both cefotaxime (30 μg) and ceftazidime (30 μg) alone and in combination with clavulanic acid in order to detect ESBL production according to the method published by the Clinical Laboratory Standards Institute (CLSI, 2012). The presence of ESBL was detected by an increase in the zone diameters (≥5 mm) of either ceftazidime or cefotaxime with clavulanic acid. For quality control, *Klebsiella pneumonia* (ATCC 700603) were used as the standard strain.

Antibiotic resistance of β-lactamase producing *E. coli*

Antimicrobial resistance of β-lactamase-producing *E. coli* were determined against several antibiotics by disc diffusion method according to the CLSI (2012). The following antibiotics were used; ampicillin (10 μg), ceftazidime (30 μg), cefotaxime (30 μg), ceftriaxone (30 μg), cefepime (30 μg), cefoxitin (30 μg), cefuroxime (30 μg), cephalothin (30 μg), aztreonam (30 μg), imipenem (10 μg), nalidixic acid (30 μg), ciprofloxacin (5 μg), chloramphenicol (30 μg), gentamicin (10 μg), streptomycin (10 μg), tobramycin (10 μg), amikacin (10 μg), kanamycin (30 μg), tetracycline (30 μg), trimethroprim–sulfamethoxazole (1.25/23.75 μg), amoxicillin–clavulanic acid (20/10 μg). For quality control, the ATCC standard strains *E. coli* (ATCC 25922) were used. Breakpoint values were selected based on the recommendations of CLSI, (2012) to interpret the resistance.

Determination of phylogenetic groups of β-lactamase producing *E. coli*

The phylogenetic analyses of ESBL and/or pAmpC producing *E. coli* isolates were carried out by multiplex PCR (Clermont and others 2000). Phylogenetic and subgroups (A0, A1, B1, $B2_2$, $B2_3$, D1, and D2) were determined according to presence or absence of the *chu*A, *yja*A genes and DNA fragrment (TSPE4) as described by Escobar-Paramo and others (2004).

Detection and characterization of β-lactamase and quinolone resistance genes

In order to detect TEM, OXA, SHV, and CTX-M type beta-lactamases, the bla_{TEM}, bla_{OXA}, bla_{SHV}, and bla_{CTX} genes were amplified by PCR as previously described (Ahmed and others 2007). To determine the exact subtypes of β-lactamase genes, PCR products were directly sequenced (Sanger sequencing) from both ends by using sequencing PCR primers (Refgen, Ankara). The sequencing data were compared with the previously published sequences in GenBank using the BLAST program (http://blast.ncbi.nlm.nih.gov/). The presence of AmpC type β-lactamese gene was tested using multiplex PCR as documented by Perez-Perez and Hanson (2002). Subsequently, the presence of bla_{CMY-2} was determined with primers in isolates that were found to be positive in multiplex PCR (Zhao and others 2001). The presence of the plasmid-mediated quinolone resistance (PMQR) genes *qnr*A, *qnr*B, *qnr*S, and *qep*A were examined by multiplex PCR and sequencing (Robicsek and others 2006). The isolates containing the *aac(6′)-Ib-cr* gene associated with resistance to quinolones were also identified by PCR and sequencing as described previously (Robicsek and others 2006). The following strains, *E. coli* J53 pMG298, which encodes both *aac(6′)-Ib-cr* and *qnr*B1, and *E. coli* TOP10 + pAT851 for *qep*A obtained from the Lahey Hospital and Medical Center and Pasteur Institute, respectively were used as positive control strains.

Statistic

All statistical analyses were done using SPSS version 20.0 (SPSS, Chicago, Ill., U.S.A.). Chi-square test was used to assess the significance in proportion of positive samples between sample types (chicken and beef) and the associations of resistance to other antimicrobials with the occurrence of ESBL or pAmpC β-lactamase and P value < 0.05 was considered significant.

Results

A total of 200 raw (chicken $n = 100$ and beef $n = 100$) meat samples were tested for the presence of β-lactamase

Table 1–Nucleotide sequence of the primers used in PCR.

Primers	Sequence (5′– 3′)	Amplicon length (bp)	Annealing temperature (°C)	Reference
*chu*A	F: ACGAACCAACGGTCAGGAT R:TGCCGCCAGTACCAAAGACA	279	60	Clermont and others 2000
*yja*A	F: TGAAGTGTCAGGAGACGCTG R:TGGAGAATGCGTTCCTCAAC	211	60	
TspE4C	F: GAGTAATGTCGGGGCATTCA R: GCGCCAACAAAGTATTACG	152	60	
bla_{TEM}	F: ATAAAATTCTTGAAGACGAAA R: GACAGTTACCAATGCTTAATC	1080	56	Ahmed and others 2007
bla_{SHV}	F: TTATCTCCCTGTTAGCCACC R: GATTTGCTGATTTCGCTCGG	797	56	
Whole bla_{SHV}	F: CGGCCTTCACTCAAGGATGTA R: GTGCTGCGGGCCGGATAAC	927	56	
bla_{OXA}	F: TCAACTTTCAAGATCGCA R: GTGTGTTTAGAATGGTGA	610	56	
Whole bla_{OXA}	F: GGCAATCCAGCCGGGGCCAA R: CGGGCCTGTTCCCGGGTTAA	891	56	
bla_{CTX-M}	F: CGCTTTGCGATGTGCAG R: ACCGCGATATCGTTGGT	551	56	
Whole bla_{CTX-M}	F: CCAGAATAAGGAATCCCATG R: GCCGTCTAAGGCGATAAAC	948	56	
16S rRNA	F:CCCCCTGGACGAAGACTGACR: ACCGCTGGCAACAAAGGATA	401	52	Wang and others 2002
bla_{CMY-2}	F: AACACACTGATTGCGTCTGAC R: CTGGGCCTCATCGTCAGTTA	1226	50	Zhao and others 2001
MOXM	F:GCTGCTCAAGGAGCACAGGAT R:CACATTGACATAGGTGTGGTGC	520	64	Perez-Perez and Hanson, 2002
CITM	F:TGG CCA GAA CTG ACA GGC AAA R: TTT CTC CTG AAC GTG GCT GGC	462	64	
DHAM	F:AACTTTCACAGGTGTGCTGGGT R:CCGTACGCATACTGGCTTTGC	405	64	
ACCM	F:AACAGCCTCAGCAGCCGGTTA R:TTCGCCGCAATCATCCCTAGC	346	64	
EBCM	F: TCGGTAAAGCCGATGTTGCGG R:CTTCCACTGCGGCTGCCAGTT	302	64	
FOXM	F:AACATGGGGTATCAGGGAGATG R:CAA AGC GCG TAA CCG GAT TGG	190	64	
*qnr*A	F: ATTTCTCACGCCAGGATTTG R: GATCGGCAAAGGTTAGGTCA	516	52	Robicsek and others 2006
*qnr*B	F: GATCGTGAAAGCCAGAAAGG R: ACGATGCCTGGTAGTTGTCC	469	52	
*qnr*S	F: ACGACATTCGTCAACTGCAA R: ACGACATTCGTCAACTGCAA	417	52	
aac(6′)-Ib-cr	F: TTGCGATGCTCTATGAGTGGCTA R: CTCGAATGCCTGGCGTGTTT	482	56	Park and others 2006

producing *E. coli*. Out of 100 raw chicken meat samples, 82 (82%) were found to be contaminated with cefotaxime-resistant *E. coli* which were all confirmed to be ESBL producer by disc combination method. In regard to β-lactamase genes, 81 out of 82 isolates were positive for $bla_{CTX-M-1}$ ($n = 39$), $bla_{CTX-M-3}$ ($n = 4$), $bla_{CTX-M-15}$ ($n = 4$), bla_{TEM-1b} ($n = 2$), bla_{SHV-12} ($n = 1$), $bla_{CTX-M-1}/bla_{TEM-1b}$ ($n = 10$), $bla_{CTX-M-1}/bla_{TEM-1b}/bla_{SHV-5}$ ($n = 1$), $bla_{CTX-M-1}/bla_{CMY-2}$ ($n = 1$) and bla_{TEM-1b}/bla_{CMY-2} ($n = 5$), $bla_{CTX-M-15}/bla_{SHV-12}$ ($n = 1$), $bla_{TEM-1b}/bla_{SHV-12}$ ($n = 1$), and bla_{CMY-2} ($n = 12$) genes (Table 2). For beef meat, 7 cefotaxime (2 μg/mL)-resistant *E. coli* isolates obtained from red meat samples ($n = 100$) and they all showed ESBL characteristics by disc combination method. Three out of 7 isolates carried following β-lactamase genes $bla_{CTX-M-3}$ ($n = 1$), $bla_{CTX-M-15}/bla_{TEM-1b}$ ($n = 1$), and $bla_{TEM-1b}/ bla_{CMY-2}$ ($n = 1$), whereas 4 phenotypic ESBL-positive strains did not harbor tested genes (Table 2). The rate of ESBL-producing *E. coli* contaminated meat was significantly higher ($P < 0.001$) in chicken (82%) than in the beef samples (7%). There were no isolates detected with bla_{OXA}. Three isolates obtained from chicken meat samples harbored the *qnr*S gene, whereas only one isolate was found to have *qnr*B. None of the identified isolates had other genes (*qnr*A and *aac(6')-Ib-cr*) tested in this study.

Among isolates obtained from chicken samples, 62.1% belonged to group D1, 10.9% to group D2, 9.7% to group A0, 7.3% to group $B2_3$, 6.1% to group B1 and 3.6% to group A1. For beef samples, distribution of phlogenetic group were as D1 ($n = 6$) and B1 ($n = 1$) (Table 2).

The antimicrobial resistance profile of 82 ESBL producing *E. coli* isolated from chicken meat is given in Figure 1 and Table 2. Briefly, all chicken isolates were found to be resistant against cefpodoxime and cepholothin. A total of 91.5% of the isolates were resistant to tetracycline. 89% and 75.6% of the isolates were found to be resistant to nalidixic acid and cefuroxime, respectively (Figure 1). All isolates were susceptible to imipenem and cefepime and only two isolates were found to be resistant to cefotetan. All seven ESBL positive *E. coli* isolated from red meat were resistant to cefpodoxime, streptomycin, cefuroxime, and cepholothine. Six isolates out of 7 were resistant to tetracycline, trimethroprim–sulfamethoxazole, and 5 were resistant

Table 2–Characteristics of ESBL/pAmpC-producing *E. coli*.

Isolates (*n*)	Phylogenetic groups	β-Lactam gene variant/s/	Resistance phenotypes[a]
Chickens			
1	D1	–[b]	AMP, CPD, KF, CXM, K, S, TOB, CN, NA, CIP, SXT, C, TE
1	A0	CMY-2	AMP, CPD, KF
1	D1	CMY-2	AMP, AMC, CPD, KF, NA
1	D2	CMY-2	AMP, CPD, FOX, KF, S, TE
1	$B2_3$	CMY-2	AMP, CPD, KF, CXM, NA, TE
1	D1	CMY-2	AMP, CPD, KF, CXM, NA, TE
1	$B2_3$	CMY-2	AMP, AMC, CPD, FOX, KF, NA, TE
1	$B2_3$	CMY-2	AMP, AMC, CPD, FOX, KF, S, CN, TE
1	$B2_3$	CMY-2	AMP, AMC, CPD, KF, K, S, NA, C, TE
1	$B2_3$	CMY-2	AMP, AMC, CPD, FOX, KF, S, NA, TE
1	D1	CMY-2	AMP, AMC, CPD, FOX, KF, S, NA, CIP, C, TE
1	A1	CMY-2	AMP, AMC, CPD, FOX, KF, K, S, NA, CIP, SXT, TE
1	$B2_3$	CMY-2	AMP, CPD, FOX, KF, CXM, K, S, TOB, CN, NA, CIP, SXT, C, TE
1	A0	CMY-2 + CTX-M-1	AMP, AMC, CPD, KF,CXM, CN, NA, CIP, SXT, TE
1	D1	CMY-2 + TEM-1b	AMP, CPD, KF, CXM, NA, TE
1	A1	CMY-2 + TEM-1b	AMP, CPD, KF, CXM, K, S, CN, NA, SXT, C, TE
1	D2	CMY-2 + TEM-1b	AMP, AMC, CPD, FOX, KF, NA, CIP, SXT, TE
1	D1	CMY-2 + TEM-1b	AMP, AMC, CPD, FOX, KF, S, CN, NA, CIP, SXT
1	D1	CMY-2 + TEM-1b	AMP, AMC, CPD, FOX, KF, CTT, S, CN, NA, CIP, SXT
1	D2	CTX-M-1	AMP, AMC, CPD, KF
1	D1	CTX-M-1	AMP, KF, CXM, NA, TE
1	D1	CTX-M-1	AMP, CPD, KF, CXM, TE
17	D1	CTX-M-1	AMP, CPD, KF, CXM, NA, TE
1	D2	CTX-M-1	AMP, CPD, KF, CXM, NA, TE
1	A1	CTX-M-1	AMP, CPD, KF, CXM, NA, TE
2	A0	CTX-M-1	AMP, CPD, KF, CXM, NA, TE
1	D1	CTX-M-1	AMP, CPD, KF, CXM, SXT, TE
1	D1	CTX-M-1	AMP, CPD, KF, CXM, S, ATM, TE
1	D1	CTX-M-1	AMP, AMC, CPD, FOX, KF, NA, TE
1	D1	CTX-M-1	AMP, CPD, KF, CXM, NA, CIP, TE
1	D1	CTX-M-1	AMP, CPD, KF, CXM, CN, NA, TE
1	D1	CTX-M-1	AMP, CPD, KF, CXM, NA, CIP, TE
1	B1	CTX-M-1	AMP, CPD, KF, CXM, K, SXT, C, TE
1	B1	CTX-M-1	AMP, CPD, FOX, KF, S, NA, CIP, TE
1	B1	CTX-M-1	AMP,CPD, KF, CXM, K, S, NA, ATM, TE
1	A0	CTX-M-1	AMP, CPD, KF, CXM, S, NA, CIP, SXT, TE
1	D1	CTX-M-1	AMP, AMC, CPD, FOX, KF, S, NA, CIP, TE
1	D1	CTX-M-1	AMP, AMC, CPD, FOX, KF, K, S, NA, C, TE
1	D1	CTX-M-1	AMP, CPD, KF, CXM, K, S, NA, SXT, ATM, TE
2	D1	CTX-M-1	AMP, CPD, KF, CXM, K, S, CN, NA, SXT, C, TE
1	D2	CTX-M-1 + TEM-1b	AMP, CPD, KF, CXM, NA, TE
1	D1	CTX-M-1 + TEM-1b	AMP, CPD, KF, CXM, NA, TE
1	A0	CTX-M-1 + TEM-1b	AMP, CPD, KF, CXM, NA, TE
1	D1	CTX-M-1 + TEM-1b	AMP, CPD, KF, CXM, S, NA, CIP, TE
1	D2	CTX-M-1 + TEM-1b	AMP, CPD, KF, CXM, K, S, NA, CIP, SXT, TE
1	D1	CTX-M-1 + TEM-1b	AMP, CPD, KF, CXM, K, CN, NA, SXT, C, TE
1	D1	CTX-M-1 + TEM-1b	AMP, CPD, KF, CXM, K, S, CN, NA, SXT, C, TE
2	D1	CTX-M-1 + TEM-1b	AMP, CPD, KF, CXM, K, S, NA, CIP, SXT, C, TE
1	D1	CTX-M-1 + TEM-1b	AMP,AMC, CPD, KF, CXM, K, NA, CIP, SXT, C, TE
1	D1	CTX-M-1 + TEM-1b + SHV-5	AMP, CPD, KF, CXM, K, S, CN, NA, SXT, C, TE
2	A0	CTX-M-3	AMP, CPD, KF, CXM, NA, CIP, SXT
1	D1	CTX-M-3	AMP, CPD, KF, CXM, S, NA, CIP, TE
1	D1	CTX-M-3	AMP, AMC, CPD, FOX, KF, CXM, S, NA, CIP, SXT, C, TE
1	D2	CTX-M-15	AMP, CPD, KF, CXM, TE
1	D2	CTX-M-15	AMP, CPD, KF, CXM, S, NA, TE
1	D1	CTX-M-15	AMP, CPD, KF, CXM, K, S, NA, SXT, TE
1	D1	CTX-M-15	AMP, CPD, KF, CXM, K, S, NA, ATM, TE
1	D2	CTX-M-15 + SHV-12	AMP, CPD, KF, CXM, K, S, NA, SXT, ATM, TE
1	D1	SHV-12	AMP, KF, S, NA, CIP, SXT, C, ATM, TE
1	B1	SHV-12 + TEM-1b	AMP, CPD, KF, CXM, K, S, NA, CIP, SXT, ATM, TE
1	B1	TEM-1b	AMP, AMC, CPD, KF, S, NA, SXT, TE
1	D1	TEM-1b	AMP, AMC, CPD, FOX, KF, CTT, K, S, TOB, NA, CIP, SXT, C, TE
Beef			
1	D1	–[b]	AMP, CPD, KF, CXM, K, S, NA, CIP, SXT, CTX, TE
1	D1	–[b]	AMP, CPD, KF, CXM, K, S, CN, NA, CIP, SXT, CTX, C
1	D1	–[b]	CPD, KF, CXM, K, S, NA, SXT, CIP, C, TE

(*Continued*)

Table 2–Continued.

Isolates (*n*)	Phylogenetic groups	β-Lactam gene variant/s/	Resistance phenotypes[a]
1	D1	–[b]	AMP, CPD, KF, CXM, K, S, CN, CIP, SXT, C, TE
1	D1	CMY-2 + TEM-1b	AMP, CPD, KF, CXM, K, S, CN, TOB, NA, SXT, C, TE
1	D1	CTX-M-3	AMP, CPD, KF, CXM, S, NA, TE
1	B1	CTX-M-15 + TEM-1b	AMP, CPD, KF, CXM, S, SXT, CTX, TE

[a]AMC, trimethroprim–sulfamethoxazole; AMP, ampicillin; CPD, cefpodoxime; FOX, cefoxitin; KF, cephalothin; CXM, cefuroxime; CTT, cefotetan; K, kanamycin; AK, amikacin; S, streptomycin; TOB, tobramycin; CN, gentamycin; NA, nalidixic acid; CIP, ciprofloxacin; CTX, cefotaxime; STX, trimethroprim–sulfamethoxazole; IPM, imipenem; C, chloramphenicol; FEB, cefepime; ATM, aztreonam; TE, tetracycline.
[b]No gene variants tested were found for these isolates.

to kanamycin and nalidixic acid. All 7 were susceptible to cefotetan, imipenem, cefoxitin, and cefepime. Statistical analyses showed that ampicillin and cefoxitin resistance profile were all significantly ($P = 0.001$) more associated with isolates that had the $bla_{\text{CMY-2}}$ gene alone. Conversely, cefuroxime and streptomycin resistance were more strongly associated with isolates having $bla_{\text{CTX-M}}$ genes ($P < 0.05$). In addition, kanamycin, ciprofloxacin, trimethroprim—sulfamethoxazole, and chloramphenicol resistance were also present in isolates ($bla_{\text{CTX-M}} + bla_{\text{TEM-1b}}$) at significantly higher level when compared with only $bla_{\text{CTX-M}}$ isolates ($P < 0.05$). However, only trimethroprim–sulfamethoxazole resistance was significantly higher in isolates having $bla_{\text{CMY-2}} + bla_{\text{TEM-1b}}$ when compared to only $bla_{\text{CMY-2}}$ ($P = 0.036$).

Discussion

Extended spectrum β-lactamase (ESBL)-producing Enterobacteriaceae, initially appeared in Europe in the 1980s, have since been an important global problem (Nathisuwan and others 2004). There have been numerous reports on isolation and molecular characterization of ESBL producing *E. coli* from food producing animals and a significant increase in the prevalence of ESBL producing *E. coli* in foods of animal origin has been also reported (Egea and others 2012). In a study conducted in the Netherlands, it is reported that there were high degree of similarity between β-lactamase genes in *E. coli* between in humans and retail meat suggesting that the possible transfer of these pathogens from animals to humans (Overdevest and others 2011). In Turkey, presence of ESBL-producing *E. coli* was reported from retail meat by Gündogan and Avci (2013). However, these authors did not study the genetic makeup of ESBLs obtained from food of animal origin. In the current study, chicken meat was found to be highly contaminated (82%) with ESBL-producing *E. coli* whereas a low prevalence of ESBL-producing *E. coli* (%7) in beef samples was found. Results obtained in the present study are not surprising when compared with other reports in which ESBL-producing *E. coli* in chicken meat to be 67% to 93.3% in Spain (Egea and others 2012; Doi and others 2009), 76.8% to 94% in the Netherlands (Leverstein-van Hall and others 2011; Overdevest and others 2011), 85% in USA (Doi and others 2009), whereas only 4.7% and 9% of beef samples were found to be positive in the Netherlands and Spain, respectively (Doi and others 2009; Overdevest and others 2011). Recently, Egervärn and others (2014) also found that the prevalence of ESBL-producing *E. coli* in chicken meat imported from South America and Europe to Sweden were 95% and 61%, respectively. Geser and others (2012) and Bortolai and others (2010) were also isolated these pathogens from broilers in Denmark and Switzerland which are the countries strictly applying antimicrobial usage rules. Geser and others (2012) attributed this fact to the couple of reasons which can be summed as 3rd and 4th generation cephalosporins are commonly used in veterinary medicine and co-selection mechanisms for multiple resistance mechanisms.

β-Lactamase (ESBL and plasmid mediated AmpC β-lactamase) enzymes have been detected in *E. coli* isolated from various foods including different retail meat samples in the world (Agersø and others 2012, Leverstain-van Hall and others 2011). To our best knowledge, this is, however, the first report of the presence of β-lactamase genes in *E. coli* isolated from retails meats in Turkey. In this study, the most frequently detected gene was $bla_{\text{CTX-M-1}}$, alone and in combination with other ESBL genes. We also detected that CTX-M-3 and CTX-M-15 type ESBLs carrying *E. coli* strains were present in chicken and beef samples. These results are consistent with previous reports in which a significant increase in *E. coli* carrying CTX-M was reported since 2000s (Livermore and others 2007). In a study conducted in United Kingdom, the

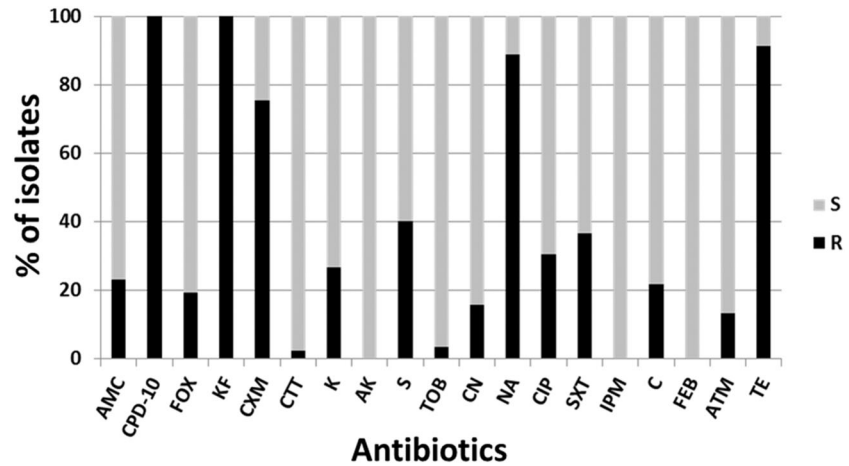

Figure 1–Antimicrobial susceptibility profile of 82 ESBL producing *E. coli* isolates recovered from chicken raw meat samples. S, susceptible; R, resistant; AMC, amoxicillin–clavulanic acid; AMP, ampicillin; CPD, cefpodoxime (10 μg); FOX, cefoxitin; KF, cephalothin; CXM, cefuroxime; CTT, cefotetan; K, kanamycin; AK, amikacin; S, streptomycin; TOB, tobramycin; CN, gentamicin; NA, nalidixic acid; CIP, ciprofloxacin; STX, trimethroprim–sulfamethoxazole; IPM, imipenem; C, chloramphenicol; FEB, cefepime; ATM, aztreonam; TE, tetracyline.

$bla_{CTX\text{-}M\text{-}1}$ gene was the most predominant ESBL type in *E. coli* isolates obtained from chicken (Perez and others 2007). A similar finding was also reported by Leverstain-van Hall and others (2011) who found that $bla_{CTX\text{-}M\text{-}1}$ was the predominant gene in *E. coli* isolated from chicken meat in the Netherlands. However, Agersø and others (2012) reported that CMY-2 was the most predominant type (48%) of enzymes among *E. coli* isolated from chicken meat samples followed by CTX-M-1 (25%) in Spain, which is not surprising as it was already reported that the proportion of *E. coli* isolates with ESBL types vary according to the geographical origins (Riaño and others 2006; Egervärn and others 2014). CTX-M-15 ESBL type was reported as the dominant type in *E. coli* isolates obtained from clinical samples in Turkey (Altınkum and others 2013) and this enzyme was found in a small number of isolates in this study. In our study, none of the isolates from meat samples were OXA type ESBL producers which are rarely reported among *Enterobacteriaceae* (Kücükbasmacı and others 2008). In addition, there has been a significant association found between the presence of ESBL genes, most notably $bla_{CTX\text{-}M\text{-}15}$ and the plasmid-mediated quinolone resistance PMQR genes (Nordman and Poirel 2005). However, there are only four isolates harbored the quinolone resistance genes in this study.

In our study, all isolates obtained from chicken and beef samples were resistant to cefpodoxime and cepholothin. Resistance to tetracycline, nalidix acid, and cefuroxime were detected in 91.5%, 89%, and 75% in chicken isolates. However, all isolates obtained from beef were found to be resistant to tetracycline and streptomycin. The results obtained in this study are similar to those of previous reports for food animal isolates (Geser and others 2012; Kücükbasmacı and others 2008). For example, in a study performed by Geser and others (2012) in Switzerland, a total of 83.5% of ESBL-producing *Enterobacteriaceae* isolated from poultry, cattle, swine and sheep were also resistant to tetracycline. In another study carried out by Kücükbasmacı and others (2008), it was shown that all ESBL producing isolates from food animals displayed resistance to tetracycline in Turkey. The imprudent use of tetracycline and quinolone in veterinary medicine may contribute to the high level of resistance. In addition, no resistance to cefepime, amikacin, and imipenem was detected in ESBL-producing *E. coli*. Imipenem is the drug of choice for serious infections by ESBL-producing *E. coli* in humans. The complete absence of imipenem resistance is probably due to not using this agent in food producing animals. In addition, it has been reported that ESBL-positive *E. coli* strains isolated from non-human primates were found to have higher resistance rates when compared with those of ESBL-negative strains (Wang and others 2012). Similarly, the rate of isolates resistance to cefoxitin, cefuroxime, and streptomycin in ESBL-producing isolates was significantly higher than in these harboring the $bla_{CMY\text{-}2}$ gene in this study. These associations were previously explained based on the carriage of antimicrobial resistance genes, particularly to aminoglycosides, by mobile genetic elements like plasmids (Wang and others 2012).

In conclusion, a high proportion of retail chicken meat samples were found to contaminated with ESBL-producing *E. coli*. In addition, majority of isolates harbored CTX-M type ESBL including CTX-M-15 suggesting that retail chicken might pose a significant hazard to the consumer. In this regard, there is, however, a need to investigate the clonal relatedness between ESBL-producing *E. coli* isolates obtained from human and food of animal origins in Turkey. It is unrealistic to expect to control ESBL-producing *E. coli* in food animals, hence in raw foods, especially those for chicken meat, so that it has to be considered that regular surveillance program should be established to monitor the antimicrobial resistance level for these organisms. It is also major importance that the use of carbapenems has to be under control in veterinary medicine.

Acknowledgments

This study received a grant from Mustafa Kemal Univ. (BAP-1207 M 0107–2012). We gratefully thank Dr. George Jacoby (Lahey Hospital and Medical Center, U.S.A.) and Prof. Dr. Patrice Courvalin (Pasteur Institute, France) for the gift of the *E. coli* strains. We are also thankful to Dr. Rafat Al Jassim (The Univ. of Queensland, Australia) for improving the manuscript.

References

Agersø Y, Aarestrup FM, Pedersen K, Seyfarth AM, Struve T, Hasman H. 2012. Prevalence of extended-spectrum cephalosporinase (ESC)-producing *Escherichia coli* in Danish slaughter pigs and retail meat identified by selective enrichment and association with cephalosporin usage. J Antimicrob Chemother 67:582–88.

Ahmed AM, Motoi Y, Sato M, Maruyama A, Watanabe H, Fukumoto Y, Shimamoto T. 2007. Zoo animals as a reservoir of Gram negative bacteria harboring integrons and antimicrobial resistance genes. Appl Environ Microb 73:6686–90.

Altinkum SM, Ergin S, Bahar H, Mamal Torun M. 2013. CTX-M-15 type extended spectrum β-lactamase producing *Escherichia coli* and *Klebsiella pneumoniae*: A developing problem in infected outpatients and hospitalised patients in Istanbul, Turkey. Afr J Microbiol Res 7:692–97.

Ben Sallem R, Ben Slama K, Saenz Y, Rojo-Bezares B, Estepa V, Jouini A, Gharsa H, Klibi N, Boudabous A, Torres C. 2012. Prevalence and characterization of extended-spectrum beta-lactamase (ESBL)- and CMY-2-producing *Escherichia coli* isolates from healthy food-producing animals in Tunisia. Foodborne Pathog Dis 9:1137–42.

Blanc V, Mesa R, Saco M, Lavilla S, Prats G, Miro E, Navarro F, Cortes P, Llagostera M. 2006. ESBL- and plasmidic class C beta-lactamase-producing *E. coli* strains isolated from poultry, pig and rabbit farms. Vet Microbiol 118:299–304.

Bonnet R. 2004. Growing Group of Extended-Spectrum-Lactamases: the CTX-M enzymes. Antimicrob Agents Chemother 48:1–14.

Bortolaia V, Guardabassi L, Trevisani M, Bisgaard M, Venturi L, Bojesen AM. 2010. High diversity of extended-spectrum β-lactamases in *Escherichia coli* isolates from Italian broiler flocks. Antimicrob Agents Chemother 54:1623–26.

Bradford PA. 2001. Extended-spectrum beta–lactamases in the 21st century: characterization, epidemiology and detection of this important resistance threat. Clin Microbiol Rev 14:933–51.

Brinas L, Moreno MA, Teshager T, Saenz Y, Porrero MC, Dominguez L, Torres C. 2005. Monitoring and characterization of extended-spectrum beta-lactamases in *Escherichia coli* strains from healthy and sick animals in Spain in 2003. Antimicrob Agents Chemother 49:1262–64

Clermont O, Bonacorsi S, Bingen E. 2000. Rapid and simple determination of the *Escherichia coli* phylogenetic group. Appl Environ Microb 66:4555–58.

Clinical and Laboratory Standards Institute (CLSI). 2012. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-second informational supplement. CLSI document M100–S22, Vol. 32 (3), Wayne, P.A.

Doi Y, Paterson DL, Egea P, Pascual A, López-Cerero L, Navarro MD, Adams-Haduch JM, Qureshi ZA, Sidjabat HE, Rodríguez-Baño J. 2009. Extended-spectrum and CMY-type **β;-**lactamase-producing *Escherichia coli* in clinical samples and retail meat from Pittsburgh, USA and Seville, Spain. Clin Microbiol Infect 16:33–38.

Egea P, López-Cerero L, Torres E, Gómez-Sánchez MC, Serrano L, Navarro Sánchez-Ortiz MD, Rodriguez-Baño J, Pascual A. 2012. Increased raw poultry meat colonization by extended spectrum beta-lactamase-producing *Escherichia coli* in the south of Spain. Int J Food Microbiol 159:69–73.

Egervärn M, Börjesson S, Byfors S, Finn M, Kaipe C, Englund S, Lindblad M. 2014. *Escherichia coli* with extended-spectrum beta-lactamases or transferable AmpC beta-lactamases and *Salmonella* on meat imported into Sweden. Int J Food Microbiol 171:8–14.

Escobar-Paramo P, Grenet K, LeMenac'h A, Rode L, Salgado E, Amorin C, Gouriou S, Picard B, Rahimy MC, Andremont A, Denamur E, Ruimy R. 2004. Large-scale population structure of human commensal *Escherichia coli* isolates. Appl Environ Microb 70:5698–700.

Geser N, Stephan R, Hächler H. 2012. Occurrence and characteristics of extendedspectrum β-lactamase (ESBL) producing *Enterobacteriaceae* in food producing animals, minced meat and raw milk. BMC Vet Res 8:21. DOI:10.1186/1746-6148-8-21.

Gundogan N, Avci E. 2013. Prevalence and antibiotic resistance of extendedspectrum beta-lactamase (ESBL) producing *Escherichia coli* and *Klebsiella species* isolated from foods of animal origin in Turkey. Afr J Microbiol Res 7:4059–64.

Kaya O, Akcam FZ, Gonen I, Unal O, Ceylan T. 2013. Risk factors for bacteremia due to extended-spectrum beta-lactamase-producing *Escherichia coli* in a Turkish hospital. J Infect Dev Ctries 7:507–12.

Kücükbasmacı O, Çiftcioglu G, Midilli K, Issa G. 2008. Detection of extended spectrum β-Lactamase producing *Enterobacteriaceae* from food animals in Turkey. Rev Méd Vét-Toulouse 159:586–92.

Jacoby GA. 2009. AmpC beta-lactamases. Clin Microbiol Rev 22:161–82.

Jouini A, Vinue L, Slama KB, Saenz Y, Klibi N, Hammami S, Boudabous A, Torres C. 2007. Characterization of CTX-M and SHV extended-spectrum beta-lactamases and associated resistance genes in *Escherichia coli* strains of food samples in Tunisia. J Antimicrob Chemoth 60:1137–41.

Leininger DJ, Roberson JR, Elvinger F. 2001. Use of eosin methylene blue agar to differentiate *Escherichia coli* from other gram-negative mastitis pathogens. J Vet Diagn Invest 13:273–75.

Leverstein-van Hall MA, Dierikx CM, Cohen Stuart J, Voets GM, Vanden Munckhof MP, VanEssen-Zandbergen A, Platteel T, Fluit AC, Vande Sande-Bruinsma N, Scharinga J, Bonten MJM, Mevius DJ. 2011. Dutch patients, retail chicken meat and poultry share the same ESBL genes, plasmids and strains. Clin Microbiol Infect 17:873–80.

Liebana E, Batchelor M, Hopkins KL, Clifton-Hadley FA, Teale CJ, Foster A, Barker L, Threlfall EJ, Davies RH. 2006. Longitudinal farm study of extended-spectrum beta-lactamase-mediated resistance. J Clin Microbiol 44:1630–34.

Livermore DM. 2008. Defining an extended-spectrum b-lactamase. Clin Microb Infect 14:3–10.

Livermore DM, Canton R, Gniadkowski M, Nordmann P, Rossolini GM, Arlet G, Ayala J, Coque TM, Kern-Zdanowicz I, Luzzaro F, Poirel L, Woodford N. 2007. CTX-M: changing the face of ESBLs in Europe. J Antimicrob Chemother 59:165–74.

Nathisuwan S, Burgess DS, Lewis JS. 2001. Extended-spectrum β-Lactamases: Epidemiology, detection, and treatment. Pharmacotherapy 21:920–28.

Nordman P, Poirel L. 2005. Emergence of plasmid-mediated resistance to quinolones in Enterobacteriaceae. J Antimicrob Chemother 56:463–69.

Overdevest I, Willemsen I, Rijnsburger M, Eustace A, Xu L, Hawkey P, Heck M, Savelkoul P, Vandenbroucke-Grauls C, Zwaluw K, Huijsdens X, Kluytmans J. 2011. Extended-spectrum β-Lactamase genes of *Escherichia coli* in chicken meat and humans, the Netherlands. Emerg Infect Dis 17:1216–22.

Park CH, Robicsek A, Jacoby GA, Sahm D, Hooper DC, 2006. Prevalence in the United States of aac(6′)-ıbr-cr encoding a ciprofloxacin-modifying enzyme. Antimicrob Agents Chemother 50:3953–5

Paterson DL, Bonomo RA. 2005. Extended-spectrum-beta-lactamases: A clinical update. Clin Microb Rev 18:657–86.

Pérez-Pérez FJ, Hanson ND. 2002. Detection of plasmid-mediated AmpC beta-lactamase genes in clinical isolates by using multiplex PCR. J Clin Microbiol 40:2153–62.

Perez F, Endimiani A, Hujer KM, Bonomo RA. 2007. The continuing challenge of ESBLs. Curr Opin Pharmacol 7:459–69.

Philippon A, Arlet G, Jacoby GA. 2002. Plasmid-determined AmpC-type beta-lactamases. Antimicrob Agents Chemother 46:1–11.

Pitout JD, Laupland KB. 2008. Extended-spectrum beta-lactamase-producing Enterobacteriaceae: an emerging public-health concern. Lancet Infect Dis 8:159–66.

Pitout JD. 2010. Infections with extended-spectrum beta-lactamase-producing *Enterobacteriaceae*: Changing epidemiology and drug treatment choices. Drugs 70:313–33.

Riaño I, Moreno MA, Teshager T, Sáenz Y, Domínguez L, Torres C. 2006. Detection and characterization of extended-spectrum beta-lactamases in *Salmonella enterica* strains of healthy food animals in Spain. J Antimicrob Chemother 58:844–47.

Robicsek A, Strahilevitz J, Sahm DF, Jacoby GA, Hooper DC. 2006. qnr prevalence in ceftazidime-resistant enterobacteriaceae isolates from the United States. Antimicrob Agents Chemother 50:2872–74.

Rupp ME, Paul D. 2003. Extended spectrum beta-lactamase (ESBL) producing *Enterobacteriaceae*. Drugs 63:353–56.

Wang GC, Clifford G, Rodgers FG. 2002. Detection in *Escherichia coli* of the genes encoding the major virulence factors, the genes defining the O157:H7 serotype, and components of the Type 2 shiga toxin family by multiplex PCR. J Clin Microbiol 40:3613–19.

Wang Y, He T, Han J, Wang J, Foley SL, Yang G, Wane S, Shen J, Wua C. 2012. Prevalence of ESBLs and PMQR genes in fecal *Escherichia coli* isolated from the non-human primates in six zoos in China. Vet Microbiol 159:53–59.

Zhao S, White DG, Mcdermott PF, Friedman S, English L, Ayers S, Meng J, Maurer JJ, Holland R, Walker RD. 2001. Identification and expression of cephamycinase bla_{CMY} genes in *Escherichia coli* and *Salmonella* isolates from food animals and ground meat. Antimicrob Agents Chemother 45:3647–50.