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ORIGINAL ARTICLE

Phenotypic and genotypic antibiotic resistance profiles of *Escherichia coli* O157 from cattle and slaughterhouse wastewater isolates

Naim Deniz Ayaz · Yilmaz Emre Gencay · Irfan Erol

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Abstract The aims of this study were to determine the minimal inhibition concentration of 20 different antibiotics on cattle and slaughterhouse wastewater *Escherichia coli* O157, including both Shiga toxigenic *E. coli* O157 (STEC O157) and non-Shiga toxigenic strains (non-STEC O157) by the Epsilometer test, and to determine the antibiotic resistance gene profiles of the isolates by PCR. A total of 102 cattle and slaughterhouse wastewater *E. coli* O157 isolates including 96 *E. coli* O157:H7$^+$ (81 non-sorbitol fermenting [NSF] STEC O157:H7, 12 NSF non-STEC O157:H7, and three sorbitol fermenting [SF] non-STEC O157:H7) and six non-STEC O157:H7$^-$ isolated from 744 cattle and slaughterhouse wastewater samples collected within a 2-year period were assessed. Of 93 NSF *E. coli* O157:H7 isolates, 19 were resistant to tetracycline and sulfamethoxazole, 14 to trimethoprim, 13 to cefoxitin, 11 to streptomycin, 10 to ampicillin, eight to chloramphenicol, six to cephalothin, four to cefaclor, four to aztreonam, and four to nalidixic acid. In six of the *E. coli* O157:H7$^-$ isolates, tetracycline resistance was detected while five of them were also resistant to ampicillin, sulfamethoxazole, and trimethoprim. In PCR analysis, 26.0 % (25/96) of the NSF *E. coli* O157:H7$^+$ and all of the *E. coli* O157:H7$^-$ isolates harbored one or more antibiotic resistance genes. While *tetA, tetB, tetC, strA, strB,* and *sulI* genes were detected from a number of the isolates, *tetD, tetE, tetG, cmlA, floR, sulII, aadA,* and *ampC* genes were not detected in any of the isolates. Results suggest a high antibiotic resistance in *E. coli* O157:H7$^+$/H7$^-$ cattle and wastewater isolates. The majority of our resistant isolates, antibacterial resistance genes did not correlate with observed phenotypic resistance. Other resistance traits and regulatory factors that mediate antibiotic resistance should be included in further antimicrobial resistance investigations.

Keywords *E. coli* O157:H7 · Antibiotic resistance · MIC · E-Test · Resistance genes · PCR

N. D. Ayaz (✉) · Y. E. Gencay
Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Kirikkale University, Yahsihan 71450, Kirikkale, Turkey
e-mail: naimdenizayaz@kku.edu.tr

I. Erol
General Directorate of Food and Control, Republic of Turkey Ministry of Food Agriculture and Livestock, Lodumlu, 06530 Ankara, Turkey

Introduction

Escherichia coli O157:H7 is considered one of the most important food-borne pathogens among Shiga toxin-producing (*stx$_1$, stx$_2$*) *E. coli* (STEC) strains. It causes diarrhea that may result in life-threatening conditions ranging from hemorrhagic colitis (HC) to hemolytic-uremic syndrome (HUS). Cattle are reported as the main asymptomatic carriers of *E. coli* O157:H7 and may disseminate the pathogen and infect humans via contaminated products or through the food chain (Meng et al. 2001). Non-STEC O157 strains can also commonly be found in cattle (Shelton et al. 2006; Durso and Keen 2007). Although the pathogenicity of non-STEC O157 isolates to humans is not clear (Durso and Keen 2007), it will be important to investigate the antibiotic resistance profiles for dissemination potential of the resistance to STEC O157 and environment.

Use of antibiotic treatment in humans for treatment of STEC infections is considered dangerous due to the lysis of cells, increased expression of *stx* genes, and release of Shiga toxins (Stx) in the intestinal tract which causes HUS (Kimmitt et al. 2000; Wong et al. 2000). However, it is reported that using some antimicrobials in the early stage of infection may be protective against HUS progression (Ikeda et al. 1999) and increased survival rates by means of rifampicin and

gentamicin use in *E. coli* O157:H7 infected mice was also reported (Rahal et al. 2011).

Bacterial infections that are resistant to antibiotics continue to cause significant health problems in humans around the world (CDC 2013). In several studies it has been reported that due to the extensive and/or inappropriate use of antibiotics in veterinary medicine for disease prevention, prophylaxis, or growth promotion in animal production, antibiotic resistance in *E. coli* and *E. coli* O157:H7 have been reported (Schroeder et al. 2004; Goncuoglu et al. 2010). To link the antibiotic use in agriculture with increased antibiotic resistant infections in humans, three scenarios have been specified (Singer and Williams-Nguyen 2014). In the first scenario, antibiotic use in agriculture may increase resistant pathogens which then infect humans via the food chain or the environment. According to the second scenario, antibiotic use may cause resistance in non-pathogenic strains which then can be transferred to pathogenic strains. In the third scenario, non-pathogenic strains gain antibiotic resistance due to antimicrobials that are released to environment and then, these strains horizontally transfer the resistance to pathogenic strains (Singer and Williams-Nguyen 2014). Additionally, the contribution of wastewater to the spread of antibiotic resistance genes to the environment (Czekalski et al. 2012; Marti et al. (2013) has been reported.

While the present study did not examine the relationship between the antibiotic use in veterinary medicine and resistance in *E. coli* O157, it may provide information about the antibiotic resistance profiles of STEC and non-STEC O157 cattle and slaughterhouse wastewater isolates, and the results of this study can be used as a starting point to examine how and if management or processing changes might impact the antibiotic resistance profiles of *E. coli* O157 from cattle and slaughterhouse wastewater. This study characterizes antibiotic resistance of *E. coli* O157 [sorbitol fermenting (SF) and non-sorbitol fermenting (NSF), STEC and non-STEC] cattle and wastewater isolates and compares the results of phenotypic and genotypic resistance.

Materials and methods

Bacterial strains

A total of 102 cattle and slaughterhouse wastewater isolates consisted of 96 *E. coli* O157:H7$^+$ (81 NSF STEC O157:H7, 12 NSF non-STEC O157:H7, and three SF non-STEC O157:H7) and six non-STEC O157:H7$^-$ isolated from 720 cattle and 24 slaughterhouse wastewater samples collected with monthly visits within a 2-year period in Kirikkale, Turkey were assessed (Ayaz et al. 2014). Out of 102 *E. coli* O157:H7$^+$/ H7$^-$, after colonies that were isolated from the same sample and harboring the same virulence gene distribution were eliminated, 29 representative subset isolates showing the exact variety of virulence gene distribution (Ayaz et al. 2014) were tested by Epsilometer test (E-test) and conventional PCR for determination of phenotypic antibiotic susceptibility and genotypic antibiotic resistance profiles, respectively. Eighty of these strains were isolated from either carcass sponge or rectoanal mucosal swab (RAMS) samples of cattle that were categorized according to age, breed, or gender, while the remaining 22 were isolated from slaughterhouse wastewater efflux. All the strains used in this study were confirmed by PCR following isolation with immunomagnetic separation based cultivation on Sorbitol MacConkey agar (Oxoid CM0813, Hampshire, UK) supplemented with Cefixime-Tellurite (Oxoid SR0172) (CT-SMAC) (Ayaz et al. 2014).

Detection of MIC values of antibiotics on *E. coli* O157:H7$^+$/H7$^-$ isolates

MIC values for 20 different antibiotics were determined by the E-test, using MIC test strips according to the manufacturer (Liofilchem MIC Test Strips, Roseto degli Abruzzi, Italy) (Table 1) and *E. coli* ATCC 25922 as the quality control strain. Cultures suspended to 0.5 McFarland standard were spread on Mueller-Hinton agar (MHA, Oxoid CM0337) and incubated at 37 °C for 18 h after the strips were placed. Following incubation, edges of the inhibition ellipse were recorded and breakpoints were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) (Anon 2006). Breakpoints for streptomycin and sulfamethoxazole were interpreted according to Centers for Disease Control and Prevention criteria (CDC 2012).

Detection of antibiotic resistance gene profiles of *E. coli* O157:H7$^+$/H7$^-$ isolates by PCR

Antibiotic resistance genes encoding tetracycline efflux pump (*tetA*, *tetB*, *tetC*, *tetD*, *tetE*, and *tetG*), streptomycin phosphotransferases (*strA* and *strB*), aminoglycoside adenyltransferase (*aadA*), chloramphenicol transporter nonenzymatic chloramphenicol-resistance protein (*cmlA*), 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 (Srinivasan et al. 2007).

DNA extraction from the isolates was carried out with Chelex-100 (Bio-Rad, Hercules, CA, USA) as previously described (Ayaz et al. 2014) and 10 μl of the resultant supernatants were used as DNA templates in PCR assays. Primer pairs (Eurofins MWG Operon, Ebersberg, Germany) used in the PCR assays were given in Table 2. Each PCR reaction of 50 μl consisted of 1 × PCR buffer, 2 mM $MgCl_2$, 0.2 mM of each dNTP, 1 U of Taq DNA polymerase (Bioron GmbH,

Table 1 Antibiotic E-test strips used to determine the antibiotic susceptibility profiles of *E. coli* O157:H7^{+}/H7^{-} cattle and wastewater isolates and antibiotic resistance patterns of the isolates

Group	Antibiotic strip	Abb.	Concentration (μg/ml)	MIC breakpoints (μg/ml)*		Number of isolates*		
				S	R	S	I	R
Aminoglycoside	Amikacin	AK	0.016-256	≤ 16	≥ 64	102	-	-
Aminoglycoside	Streptomycin	S	0.064-1024	≤ 32	≥ 64	91	-	11
Aminoglycoside	Tobramycin	TOB	0.064-1024	≤ 4	≥ 16	98	4	-
Aminoglycoside	Gentamicin	CN	0.016-256	≤ 4	≥ 16	102	-	-
Aminoglycoside	Kanamycin	K	0.016-256	≤ 16	≥ 64	91	11	-
Penicillins	Ampicillin	AMP	0.016-256	≤ 8	≥ 32	82	5	15
β-Lactam/(ase) inhibitor	Amoxicillin/clavulanic acid	AUG	0.016-256	≤ 8/4	≥ 32/16	89	13	-
Monobactams	Aztreonam	ATM	0.016-256	≤ 8	≥ 32	98	-	4
Cephalosporin 1st gen.	Cephalothin	KF	0.016-256	≤ 8	≥ 32	54	42	6
Cephalosporin 2nd gen.	Cefaclor	CEC	0.016-256	≤ 8	≥ 32	98	-	4
Cephalosporin 2nd gen.	Cefoxitin	FOX	0.016-256	≤ 8	≥ 32	85	4	13
Cephalosporin 3rd gen.	Cefotaxime	CTX	0.016-256	≤ 8	≥ 64	102	-	-
Cephalosporin 3rd gen.	Ceftriaxone	CRO	0.016-256	≤ 8	≥ 64	91	11	-
Fluoroquinolone 1st gen.	Norfloxacin	NOR	0.016-256	≤ 4	≥ 16	91	11	-
Fluoroquinolone 2nd gen.	Ciprofloxacin	CIP	0.002-32	≤ 1	≥ 4	91	11	-
Quinolone 1st gen.	Nalidixic acid	NA	0.016-256	≤ 16	≥ 32	98	-	4
Folate Pathway Inhibitors	Sulfamethoxazole	SMX	0.064-1024	≤ 256	≥ 512	78	-	24
Folate Pathway Inhibitors	Trimethoprim	TM	0.002-32	≤ 8	≥ 16	83	-	19
Phenicols	Chloramphenicol	C	0.016-256	≤ 8	≥ 32	87	7	8
Tetracyclines	Tetracycline	TE	0.016-256	≤ 4	≥ 16	77	-	25

* **S,** Susceptible; **I,** Intermediate resistant; **R,** Resistant

Ludwigshafen, Germany) and 0.1 μM of each primer pairs. After an initial denaturation at 94 °C for 5 min, thermal cycler (Eppendorf mastercycler gradient, Hamburg, Germany) conditions were as follows: 30 cycles of denaturation at 94 °C for 45 s, annealing (temperatures as listed in Table 2) for 45 s and extension at 72 °C for 45 s. Following a final extension for 5 min at 72 °C, resultant PCR products were resolved and visualized on gels as described by Ayaz et al. (2014).

Statistical analysis

The statistical analysis for determination of the significance of age, gender, and breed of cattle on the antibiotic resistance profiles of *E. coli* O157:H7 isolates was performed with chi-square and binary logistic regression (SPSS, version 16.0).

Results

Phenotypic antibiotic resistance profiles of *E. coli O157:H7^{+}/H7^{-}* isolates

In the study, 25 of 96 *E. coli* O157:H7 and all six *E. coli* O157:H7^{-} isolates were resistant to at least one of the antibiotics tested (Tables 1 and 3). Resistance against tetracycline, sulfamethoxazole, trimethoprim, and ampicillin, were the most prevalent resistances observed, while none of the isolates showed resistance against cefotaxime, gentamicin, or amikacin. Furthermore, high prevalence (41.2 %) of intermediate resistance was observed against cephalothin, followed by amoxicillin/clavulanic acid combination (12.7 %), kanamycin (10.8 %), ceftriaxone (10.8 %), norfloxacin (10.8 %), ciprofloxacin (10.8 %), chloramphenicol (6.9 %), ampicillin (4.9 %), tobramycin (3.9 %), and cefoxitin (3.9 %). None of the 3 SF *E. coli* O157:H7 strains showed resistance to the antibiotics tested.

Five different phenotypic multiple antibiotic resistance profiles were assessed. Seven of the 96 *E. coli* O157:H7 isolates were resistant to ampicillin, cefoxitin, streptomycin, tetracycline, sulfamethoxazole, and trimethoprim, while five were resistant to tetracycline and sulfamethoxazole; four were resistant to cephalothin, cefoxitin, cefaclor, aztreonam, streptomycin, tetracycline, nalidixic acid, sulfamethoxazole, trimethoprim, and chloramphenicol; three were resistant to ampicillin, tetracycline, sulfamethoxazole, and trimethoprim; two were resistant to cephalothin and cefoxitin. In five of the six *E. coli* O157:H7^{-} isolates multiple antibiotic resistance to

Table 2 Primer pairs, annealing temperatures and PCR protocols used to determine the antibiotic resistance gene profile of *E. coli* O157:H7$^+$/H7$^-$ cattle and wastewater isolates

Antibiotic	Gene	Annealing (°C)	Nucleotide Sequence (5 - 3)	Product (bp)	Reference
Ampicillin	*ampC*	53	TTCTATCAAMACTGGCARCC[a] CCYTTTTATGTACCCAYGA[a]	550	Schwartz et al. (2003)
Tetracycline	*tetA*	58	GGCCTCAATTTCCTGACG AAGCAGGATGTAGCCTGTGC	372	Guillame et al. (2000)
	tetB	58	GAGACGCAATCGAATTCGG TTTAGTGGCTATTCTTCCTGCC	228	Guillame et al. (2000)
	tetC	58	TGCTCAACGGCCTCAACC AGCAAGACGTAGCCCAGCG	379	Guillame et al. (2000)
	tetD	58	GGATATCTCACCGCATCTGC CATCCATCCGGAAGTGATAGC	436	Guillame et al. (2000)
	tetE	58	TCCATACGCGAGATGATCTCC CGATTACAGCTGTCAGGTGGG	442	Guillame et al. (2000)
	tetG	58	CAGCTTTCGGATTCTTACGG GATTGGTGAGGCTCGTTAGC	844	Gebreyes and Altier (2002)
Florphenicol	*floR*	55	TATCTCCCTGTCGTTCCAG AGAACTCGCCGATCAATG	399	Keyes et al. (2000)
Chloramphenicol	*cmlA*	62	CCGCCACGGTGTTGTTGTTATC CACCTTGCCTGCCCATCATTAG	698	Gebreyes and Altier (2002)
Sulphisoxazole	*sulI*	58	GTGACGGTGTTCGGCATTCT TCCGAGAAGGTGATTGCGCT	779	Lanz et al. (2003)
	sulII	58	CGGCATCGTCAACATAACCT TGTGCGGATGAAGTCAGCTC	721	Lanz et al. (2003)
Streptomycin	*strA*	56	CTTGGTGATAACGGCAATTC CCAATCGCAGATAGAAGGC	548	Gebreyes and Altier (2002)
	strB	54	ATCGTCAAGGGATTGAAACC GGATCGTAGAACATATTGGC	509	Gebreyes and Altier (2002)
Aminoglycosides	*aadA*	60	GTGGATGGCGGCCTGAAGCC AATGCCCAGTCGGCAGCG	525	Lanz et al. (2003)

[a] According to the *IUPAC (International Union of Pure and Applied Chemistry)*: M, A or C; R, A or G; Y, C or T

ampicillin, tetracycline, sulfamethoxazole, and trimethoprim was detected while the remaining one was only resistant to tetracycline.

When the origin of the isolates was considered, resistance against 10 different antibiotics was observed in four of the 22 slaughterhouse wastewater isolates, which was also the widest antibiotic resistance pattern compared to the remaining of the isolates (Table 3). Of the 30 *E. coli* O157:H7$^+$/H7$^-$ originating from RAMS samples of young cattle, eight were resistant to four (ampicillin, tetracycline, sulfamethoxazole, trimethoprim) and three were resistant to six (ampicillin, cefoxitin, streptomycin, tetracycline, sulfamethoxazole, trimethoprim) antibiotics tested. No statistically significant ($p>0.05$) influence of age, gender, or breed was observed on antibiotic resistance profiles of the isolates.

Antibiotic resistance gene profiles of ***E. coli*** O157:H7$^+$/H7$^-$ isolates

A total of 102 cattle and slaughterhouse wastewater isolates including 96 *E. coli* O157:H7$^+$ (93 NSF and three SF) and six *E. coli* O157:H7$^-$ were tested by PCR to determine the presence of antibiotic resistance genes (*tetA*, *tetB*, *tetC*, *tetD*, *tetE*, *tetG*, *strA, strB, aadA, cmlA, floR, sulI, sulII*, and *ampC*). According to PCR results, 26.0 % (25/96) of the *E. coli* O157:H7$^+$ and all (6/6) of the *E. coli* O157:H7$^-$ isolates harbored one or more antibiotic resistance genes. However, none of the three SF *E. coli* O157:H7$^+$ harbored any of the antibiotic resistance genes investigated. While at least one of the *tetA, tetB, tetC, strA, strB*, and *sulI* genes were detected from 30.4 % (31/102) of the isolates (Fig. 1), *tetD, tetE, tetG, cmlA, floR, sulII, aadA*, and *ampC* genes were not detected (Table 3).

Out of 102 *E. coli* O157:H7$^+$/H7$^-$ isolates, 26 (25.5 %) were carrying at least one of the tested tetracycline resistance genes. Among tetracycline resistance genes, *tetC* was the most prevalent (14.7 %; 15/102) followed by *tetA* (12.7 %; 13/102) and *tetB* (4.9 %; 5/102). In seven of the 102 (6.9 %) isolates, both *tetA* and *tetC* genes were observed. Other than tetracycline resistance gene, *sulI*, *strA*, and *strB* were detected from 13 (12.7 %), five (4.9 %), and five (4.9 %) of the isolates, respectively.

Table 3 Phenotypic and genotypic antibiotic resistance patterns of *E. coli* O157:H7^{+}/H7^{-} cattle and wastewater isolates

Strain[a]	n	Season[b]	Age[c]	Breed[d]	Gender[e]	Phenotypic antibiotic resistance patterns[g] I	Genotypic antibiotic resistance patterns R	
Cattle isolates								
3R (A-C)	3	S	Y	N	M	AUG, KF, CRO, K, CIP, NOR, C	AMP, FOX, S, TE, SMX,TM	*tetA, tetC, sulI*
3 K (A,C,D,E)	4	S	Y	N	M	AUG, KF, CRO, K, CIP, NOR, C	AMP, FOX, S, TE, SMX,TM	*tetA, tetC, sulI*
19R (A-E)[h,i]	5	S	Y	C[1]	M		AMP, TE, SMX, TM	*tetA, sulI*
25 K (A-C)[f,h]	3	S	Y	C[2]	M			
34R (A-E)	5	S	Y	C[1]	M	KF		
36 K (A-C)	3	S	O	N	F	KF		
44R (A-D)	4	W	O	Cr	F			
44 K (A-E)	5	W	O	Cr	F			
68R (A, B)	2	W	Y	C[1]	M			
69R (A-E)	5	W	Y	Cr	M			
91 K (A-D)	4	W	Y	N	M			
120R (A-C)	3	S	Y	Cr	F			
120 K (A,B,D,E)	4	S	Y	Cr	F	KF		
120 K (C)	1	S	Y	Cr	F			
135R (A-D)	4	S	Y	C[1]	M			
143R (A-E)	5	S	M	Cr	M	KF		
143 K (A-D)	4	S	M	Cr	M			
163 K (A-E)	5	W	M	N	F		TE, SMX	*tetB, strA*
168 K (A-E)	5	W	Y	C[3]	M			*strB*
210K(B)[h,i]	1	W	M	C[3]	F	AMP, KF	TE	*tetA, sulI*
219R (A-C)	3	W	Y	Cr	M	KF	AMP, TE, SMX, TM	
236 K (B)[h]	1	S	O	N	F	AUG	KF, FOX	
236 K (E)	1	S	O	N	F	AUG	KF, FOX	
Wastewater isolates								
M1 (A)	1					AMP, AUG, CRO, K, TOB, CIP, NOR	KF, FOX, CEC, ATM, S, TE, NA, SMX, TM, C	*tetC*
M1 (C, D, E)[h]	3					AMP, AUG, CRO, K, TOB, CIP, NOR	KF, FOX, CEC, ATM, S, TE, NA, SMX, TM, C	*tetC*
M14 (A-D)[h]	4					KF, FOX	C	
M17 (A-E)	5					KF		
M18 (A-E)	5					KF		
M21 (A,C,D,E)[h]	4							*tetC*

[a] Sampled cattle number; sample (**R,** rectoanal mucosal swab; **K,** carcass sponge); **A-E,** colony code. [b] **S,** Warm months (May-October); **W,** Cold months (November-April).

[c] **Y,** Young (Y≤24 months); **M,** Mature (24 months<M≤4 years,; **O:** Old (4 years<O). [d] **C,** Culture (C[1] , Holstein; C[2] . Brown Swiss; C[3] , Simmental); **Cr,** Crossbred; **N,** Native (Anatolian Black). [e] **M,** Male; **F,** Female. [f] Sorbitol fermentative colonies on CT-SMAC (Ayaz et al. 2014). [g] **I,** Intermediate resistant; **R,** Resistant. [h] non-Shiga toxigenic isolates. [i] *E. coli* O157:H7^{-} isolates

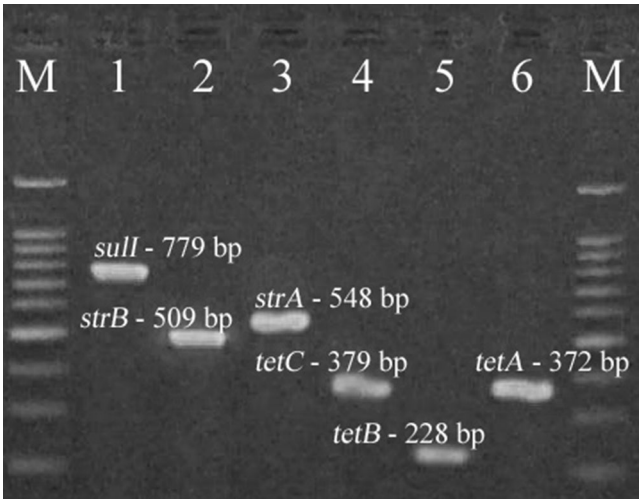

Fig. 1 Agarose gel electrophoresis of detected antibiotic resistance gene DNA fragments amplified by PCR from selected isolates. **Lane M:** 100 bp DNA marker; **Lane 1:** *E. coli* O157:H7 210 KB (*sulI* - 779 bp); **Lane 2:** *E. coli* O157:H7 168KA (*strB* - 509 bp); **Lane 3:** *E. coli* O157:H7 163KA (*strA* - 548 bp); **Lane 4:** *E. coli* O157:H7 3KA (*tetC* - 379 bp); **Lane 5:** *E. coli* O157:H7 163KA (*tetB* - 228 bp); **Lanes 6:** *E. coli* O157:H7 19RA (*tetA* – 372 bp)

Two different genotypic multiple antibiotic resistance gene profiles were detected from *E. coli* O157:H7$^+$ isolates: seven (6.9 %) harboring *tetA*, *tetC*, and *sulI*, and five (4.9 %) harboring *tetB* and *strA*. In all six (6.1 %) *E. coli* O157:H7$^-$ isolates, *tetA* and *sulI* genes were detected.

Discussion

In this study, 31 of the 102 *E. coli* O157:H7$^+$/H7$^-$ isolates originating from cattle and slaughterhouse wastewater were found to be resistant to one or more antibiotics that were investigated. Similar to this study, aztreonam, cefaclor, ampicillin, tetracycline, cephalothin, sulfamethoxazole, trimethoprim, streptomycin, and nalidixic acid resistant *E. coli* O157:H7 cattle isolates have been reported worldwide (Khan et al. 2002; Vali et al. 2004; Wilkerson et al. 2004; Srinivasan et al. 2007; Goncuoglu et al. 2010). Furthermore, a high prevalence of tetracycline, sulfonamide and ampicillin resistance was observed in accordance with a retrospective previous report that showed increased resistance against these antibiotics in *E. coli* strains, suggesting a worldwide emergence of this trend (Tadesse et al. 2012). High prevalence of resistance against these antibiotics might not be surprising as ampicillin, due to its broad spectrum activity, tetracycline, due to its broad spectrum activity and short-acting nature, and sulfonamides, due to their low cost and relative high efficacy, are among the most preferred and widely used antimicrobial approaches in veterinary medicine. However, it is important to stress that the strains in this study, which have already established resistance against such widely used antibiotics, also showed intermediate resistance towards cephalosporins such as cephalothin and ceftriaxone or clavulanate-potentiated amoxicillin, or even fluoroquinolones and chloramphenicol. Nevertheless, there was no resistance (MIC values of 0.25 - 4 μg/μl) observed against gentamicin, which has been proven to be a good candidate in the treatment of STEC O157:H7 infections (Rahal et al. 2011).

In a previous study, 63.6 %, 63.6 %, and 9.1 % *E. coli* O157:H7 isolates from cattle were found to be intermediately resistant to ampicillin, sulfamethoxazole, and cefoxitin, respectively as determined by disc diffusion Goncuoglu et al. 2010). However, we observed high prevalence of resistance to these antibiotics (sulfamethoxazole 23.5 %, ampicillin 14.7 %, cefoxitin 12.7 %). If measures to curb antibiotic resistance are not taken, it is intriguing to speculate that emergence of *E. coli* O157:H7 strains showing resistance against a wide array of antimicrobial groups is highly possible. Marti et al. (2013) showed the spread of antibiotic resistance genes to the environment via wastewater and its effect on the bacterial population of the receiving river. In a different study, selection of multiresistant strains through wastewater treatment and accumulation of resistance genes were reported (Czekalski et al. 2012). In a study by Mantz et al. (2013), high and low manure accumulation feedlot surface samples were compared for the incidence of *erm*(B) genes. According to the results, a correlation was not found between high manure accumulation and *erm*(B) distribution. In the current study, one interesting example was found with the wastewater isolates (M1; Table 3) where resistance against 10 and intermediate resistance against seven antibiotics was shown. In two studies, no relationship was found between antimicrobial use and presences of the β-lactamase gene $bla_{CTX\text{-}M}$ in swine finnishing barns and dairy cattle (Mollenkopf et al. 2012; 2013). However, in a different study, high levels of MLS_B resistance was detected from a swine farm which tetracycline antimicrobials were detected in manure samples while low levels of MLS_B resistance was found from antimicrobial-free farms (Zhou et al. 2009).

Even though the use of chloramphenicol in animals of food value was banned in Turkey in 2002 (Anon 2002), this study found that eight (8.1 %) wastewater isolates showed resistance and seven (7.1 %) cattle isolates showed intermediate resistance to this antibiotic. Pakpour et al (2012) reported that after 2.5 years since antibiotic usage has been banned, bacterial antibiotic resistance to chlortetracycline (tet_R) and tylosin (tyl_R) genes were still detected at a swine complex. Since there had been no data on chloramphenicol resistance in *E. coli* O157:H7 before the ban in Turkey, it was not possible to make a comparison with the previous occurrence. However, this finding could be attributed either to the selective pressure resulting from the illegal use of this drug or the lingering of this resistance in the population following the ban. Either way,

it is clear that slaughterhouses play an important role in dissemination of this and other resistance traits, and it is critical that governmental authorities ensure establishment of slaughterhouse wastewater treatment facilities.

In our study *tetA, tetB, tetC, strA, strB*, and *sulI* genes were detected from a number of the isolates while *tetD, tetE, tetG, cmlA, floR, sulII, aadA,* and *ampC* genes were not found. Among *tet* genes, *tetC* was the most prevalent marker (14.7 %; 15/102) followed by *tetA* (12.7 %; 13/102) and *tetB* (4.9 %; 5/102). In a different study, *tetA*, *tetB*, *tetC,* and *tetG* were found, while *tetD* and *tetE* genes were not detected from any of the *E. coli* O157:H7 isolates (Van kirk and Roberts 2004). Also, Srinivasan et al. (2007) did not detect *tetB*, *tetD*, *tetE*, and *tetG* from STEC O157:H7 isolates. With the exception of four wastewater isolates (M21; Table 3) that were harboring *tetC*, all of the isolates that carry one of the *tet* genes were found phenotypically resistant to tetracycline indicating presence of other determinants (Chopra and Roberts 2001). In accordance with Tuckman et al. (2007), no correlation of phenotypic tetracycline MIC values and presence of genotypic resistance traits could be determined in our study.

Of 24 sulfamethoxazole resistant isolates, half were harboring *sulI* and none harbored the *sulII* gene, while one strain that harbors *sulI* did not display phenotypic resistance. The resistance in isolates that lack both *sulI* and *sulII* might be attributed to single amino acid mutations that are prevalently found in *E. coli* (Lanz et al. 2003); however, presence of a transferable sulfonamide resistance trait, *sulI*, in half of the isolates displaying resistance to sulfamethoxazole is of importance. Previous work has associated ampicillin resistance with the presence of chromosomal cepholosporinase *ampC*. Srinivasan et al. (2007) have found that 71.2 % of STEC O157:H7/H7$^-$ ampicillin resistant isolates carried *ampC*; however, we did not detect *ampC* in any of the 15 ampicillin resistant *E. coli* O157:H7/H7$^-$ isolates. Observed phenotypic resistance and intermediate resistance against ampicillin, amoxicillin/clavulanic acid, and cephalosporins suggests the presence of other plasmid-mediated β-lactamases, and thus further work should investigate such traits as well (Brinas et al. 2002).

Interestingly, isolates (3R and 3 K [96 μg/μl] and M1 [384 μg/μl]) showing streptomycin resistance did not harbor any of the most common streptomycin resistance conferring genes investigated. However, only when streptomycin resistance in isolates was interpreted according to a previously recommended lower cut-off value (Sunde and Norström 2005) rather than CDC criteria (≥64 μg/μl; CDC 2012), five strains (163 K [32 μg/μl]) harboring *strA* could correlate with phenotypic resistance. In a study that investigated the accuracy of the streptomycin epidemiological cut-off value for *Escherichia coli*, 208 *E. coli* isolates exhibiting MICs between 4 and 32 mg/l were selected from 12 countries for the detection of *aadA*, *strA*, and *strB* streptomycin resistance genes by PCR. In the study, 3.6 %, 17.6 %, 53 %, and 82.3 % of the *E. coli* isolates, which were exhibiting MICs of 4 mg/l, 8 mg/l, 16 mg/l, and 32 mg/l, respectively, were not carrying the mentioned resistance genes. According to the European Committee on Antimicrobial Susceptibility Testing guidelines (cut-off value ≤16 mg/l), 25 % of the *E. coli* strains presenting MIC ≤16 mg/l would have been categorized incorrectly. Based on these results, the authors recommended a cut-off value of ≤8 mg/l for *E. coli* (Garcia-Migura et al. 2012). Even though strains harboring *strA-strB* and *aadA* were associated with high levels of streptomycin resistance (Sunde and Norström 2005), and the presence of both *strA* and *strB* was believed to be crucial for a functional streptomycin resistance (Lanz et al. 2003), our results clearly show that there are other mechanisms, such as mutations or biochemical resistance mechanisms, that might mediate streptomycin resistance as previously discussed (Garcia-Migura et al. 2012).

In conclusion, the current study shows that cattle and slaughterhouse wastewater are reservoirs of antibiotic resistant *E. coli* O157. According to the results, phenotypic antibiotic resistance and resistance genes were detected both in STEC and non-STEC O157 isolates. Appropriate control should be implemented by governmental authorities to the usage of antibiotics in veterinary medicine to curb the development of novel resistant strains. Slaughterhouse wastewater can contribute to dissemination of antibiotic resistant STEC and non-STEC *E. coli* O157 into the environment. Our data *i)* clearly demonstrated that there can be differences between resistances when measured by phenotypic and genotypic methods, and *ii)* highlighted the need of using both methods for conducting antibiotic resistance monitoring.

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