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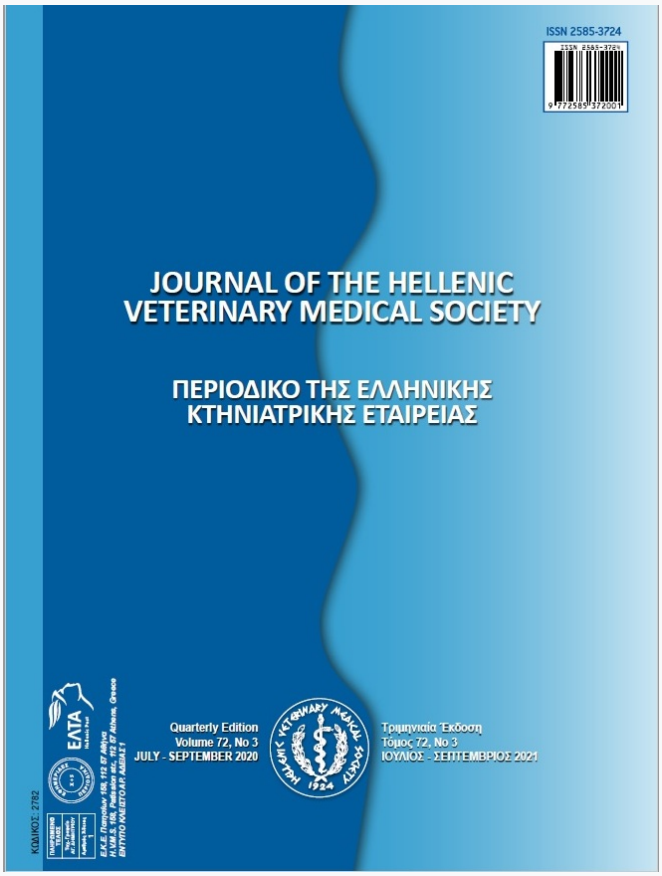

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Research article
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Screening of AmpC-/ESBL-producing *Escherichia coli* isolates from livestock for STEC/EHEC virulence genes

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ABSTRACT: Livestock is an important reservoir of Shiga toxin-producing *Escherichia coli* and enterohemorrhagic *E. coli* (STEC/EHEC) strains and acts as a significant source of transmission to humans. In addition to the virulence of STEC/EHEC isolates, antibiotic resistance is also an escalating problem in these bacteria and increases the risk to public health. Therefore, the present study aimed to explore *E. coli* O_{157}:H_7 serotype and STEC/EHEC virulence genes in AmpC- and extended-spectrum beta-lactamase (ESBL)-producing *E. coli* isolates from cattle, chicken and sheep. A total of 61 confirmed AmpC- or ESBL-producing *E. coli* isolates were screened for the virulence genes (*stx1, stx2, eae*, *ehxA, espP*, *katP* and *saa*) and *E. coli* O_{157} (*rfbO*$_{157}$) and H_7 (*fliCH*$_7$) genes by polymerase chain reaction (PCR). None of the ESBL-producing *E. coli* was positive for these genes, but six multidrug-resistant AmpC-producing *E. coli* were positive for the *fliCH*$_7$ gene only. When considering the function of the H_7 flagellar antigen of *E. coli*, it may be concluded that the development of ESBL/AmpC beta-lactamase production in the *E. coli* isolates with H_7 flagella, which reside in the chicken intestine, may be potentially important for public health regarding both virulence and antimicrobial resistance.

Keywords: AmpC, ESBL, *Escherichia coli*, *fliCH*$_7$, multidrug resistant

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INTRODUCTION

Escherichia coli is an important bacterial species containing both commensal strains of intestinal microflora and pathogenic strains causing infections in various parts of the body of both humans and animals. Therefore, *E. coli* strains are divided into two main pathogenic groups: intestinal and extraintestinal pathogenic strains. Intestinal pathogenic strains in humans have been classified into several pathotypes based on their virulence characteristics and infection mechanisms. Six main intestinal pathogenic *E. coli* strains have been described; namely,enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), enteroaggregative *E. coli*(EAEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), and diffusely adherent *E. coli* (DAEC). Intestinal pathogenic *E. coli* strains originate from domestic animals and are transmitted to humans by contaminated food, water, or by direct contact (Haiko and Westerlund-Wikström, 2013). EHEC strains are responsible for bloody diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome in humans (Bugarel et al., 2011). All of them cause life-threatening infections in humans. Livestock is known as a reservoir of STEC/EHEC strains, and cattle are notably recognized as carriers of O_{157}:H_7 and STEC/EHEC strains as revealed by several studies (Venegas-Vargas et al., 2018).

The World Health Organization (WHO) reported that extended-spectrum beta-lactamase- (ESBL) producing *E. coli* are resistant bacteria that have been classified as presenting a high risk to public health (WHO, 2017). Reports indicate an increasing prevalence of ESBL-/AmpC-producing *E. coli* as both commensal and pathogenic strains in livestock. On the other hand, the zoonotic potential of ESBL-/AmpC-producing *E. coli* (from farm animals to humans) has been proved (Huijbers et al., 2014). Therefore, it is worth investigating the virulence potentials of ESBL- and AmpC-producing *E. coli* strains isolated from healthy animals. In this study, the presence of serotype O_{157}:H_7 and STEC/EHEC virulence genes were investigated in ESBL-/AmpC-producing fecal *E. coli* isolates from cattle, chicken and sheep.

MATERIALS AND METHODS

In the present investigation, a total of 61 AmpC- or ESBL-producing *E. coli* stock isolates from previous studies were used. The above isolates originated from fecal samples of cattle, chicken and sheep in Burdur, Turkey were subjected to ESBL confirmatory test (phenotypically) according to the Clinical and Laboratory Standards Institute (CLSI) guidelines while the presence of genes (TEM, SHV and CTX-M), plasmidic AmpC genes (ACC, CIT, DHA. EBC. FOX and MOX families) and the phylogenic group(A, B1, B2, and D)were detected by PCR.The AmpC-producing *E. coli* isolates had been isolated from 2 chicken farms and ESBL-producing *E. coli* isolates had been isolated from 8 cattle, 3 sheep and 2 chicken farms (Pehlivanoglu et al., 2016; Pehlivanoglu et al., 2017; Pehlivanoglu 2017). Information about the source and phylogenetic characteristics of the isolates are presented in Table 1.

The presence of *E. coli* serotype O_{157}:H_7 and STEC/EHEC virulence genes were determined by PCR. PCR was performed for *rfbO*$_{157}$, *stx1* (Shiga toxin 1), *stx2* (Shiga toxin 2), *eae* (intimin),and *ehxA* (enterohemolysin) according to Bai et al. (2012), for *espP* (extracellular serine protease) and *katP* (catalase peroxidase) according to Posse et al. (2007),for *saa* (autoagglutinating adhesin) genes according to Paton and Paton (2002), and for *fliCH*$_7$gene according to Osek (2003). Primer sequences used in the determination of virulence genes are presented in Table 2. PCR products were run on a 1.0 % agarose gel, visualized, and photographed under UV light.

RESULTS

In the present study, 46 ESBL-producing *E. coli* were not carriers of the STEC/EHEC virulence genes investigated. Amongst the AmpC beta-lactamase-producing *E. coli* isolates (n = 15), six isolates that had been isolated from chickens were positive for the *fliCH*$_7$ gene only. The other genes investigated were also absent in the AmpC-producing *E. coli* isolates. All the *fliCH*$_7$ gene-positive *E. coli* isolates (n = 6) were from B1 phylogenetic group and one chicken flock. The antibiotic susceptibility pattern was the same for six of them, and they were multidrug-resistant (MDR) isolates (resistant to streptomycin, sulfamethoxazole-trimethoprim, nalidixic acid, enrofloxacine, and tetracycline). All the *fliCH*$_7$-positive *E. coli* isolates were CIT type pAmpC beta-lactamase producers (Table 3).

Table 1. Origins of *E. coli* isolates (Pehlivanoglu et al. (2016); Pehlivanoglu et al. (2017); Pehlivanoglu (2017).

Animal species	Beta- lactamase type	Herd / flock (n)	*E. coli* isolates (n)	Phylogenetic group						
				A_0	A_1	B1	$B2_2$	$B2_3$	D_1	D_2
Cattle	ESBL	8	31	2	15	8	-	-	3	3
Chicken	ESBL	2	12	-	-	5	-	-	1	6
Chicken	AmpC	2	15	-	8	6	-	-	1	-
Sheep	ESBL	3	3	-	2	1	-	-	-	-
Total		15	61	2	25	20	-	-	5	9

n: number of isolates

Table 2. The primer sequences for STEC/EHEC virulence genes and O_{157}:H_7 type of *E. coli*.

Target gene	Primer sequence (5'----------3')	Amplicon (bp)	Reference
$rfbO_{157}$	F-CAGGTGAAGGTGGAATGGTTGTC R-TTAGAATTGAGACCATCCAATAAG	296	Bai et al. (2012)
fliCH_7	F-GCTGCAACGGTAAGTGAT R-GGCAGCAAGCGGGTTGGT	948	Osek (2003)
stx1	F-TGTCGCATAGTGGAACCTCA R-TGCGCACTGAGAAGAAGAGA	655	Bai et al. (2012)
stx2	F-CCATGACAACGGACAGCAGTT R-TGTCGCCAGTTATCTGACATTC	477	Bai et al. (2012)
eae	F-CATTATGGAACGGCAGAGGT R-ACGGATATCGAAGCCATTTG	375	Bai et al. (2012)
ehxA	F-GCGAGCTAAGCAGCTTGAAT R-CTGGAGGCTGCACTAACTCC	199	Bai et al. (2012)
espP	F-GATTACAGCACGCATTCATGGTAT R-TCCAGGCATCCTCAGTGACA	73	Posse et al. (2007)
katP	F-GAAGTCATATATCGCCGGTTGAA R-GTCATTTCAGGAACGGTGAGATC	73	Posse et al. (2007)
saa	F-CGTGATGAACAGGCTATTGC R-ATGGACATGCCTGTGGCAAC	119	Paton and Paton, (2002)

Table 3. PCR results for STEC/EHEC virulence genes and O_{157}:H_7 type specific genes

Animal	*E. coli*isolates	n	STEC/EHEC virulence genes and O_{157}:H_7 serotype spesific genes (n)								
			rfbO$_{157}$	*fliCH*$_7$	*stx1*	*stx2*	*eae*	*ehxA*	*espP*	*katP*	*saa*
Cattle	ESBL	31	-	-	-	-	-	-	-	-	-
Chicken	AmpC	15	-	6	-	-	-	-	-	-	-
	ESBL	12	-	-	-	-	-	-	-	-	-
Sheep	ESBL	3	-	-	-	-	-	-	-	-	-

n: number of isolates

DISCUSSION

E. coli contains peritrichous flagella. The flagellum of *E. coli* has a heterogeneous character, and *E. coli* strains are classified into H-serotypes according to the seroreactivity of the variable antigenic domain of FliC (Haiko and Westerlund-Wikström, 2013). In this investigation, we detected only one gene, the-*fliCH*$_7$ gene, coding for the H_7 type flagella. Therefore, our discussion focused on the H_7 type flagella.

So far, 53 H flagellar antigens (numbered from 1 to 56, excluding 13, 22, and 50) were characterized serologically from *E. coli* species (Wang et al., 2003). On the other hand, molecular identification of the flagellum type of *E. coli* is based on the sequence of the *fliC* gene, which encodes the flagellar filament protein. Differences in the amino acid sequence in the central part of the FliC protein determine the different H types because the N and C terminal parts of the FliC protein are highly conserved, and the central part is exposed to the surface and is highly variable (Reid et al., 1999). In this study, primers specific to the H_7

type FliC protein were used. Therefore, we could determine the *E. coli* strain(s) carrying the H_7 type flagella.

The flagella of *E. coli* have been shown to play essential roles in motility and adhesion. Especially in intestinal pathogenic *E. coli* strains, H_7 flagella act as adhesins at the initiating step of EHEC infections but did not have any functions during later phases. In O_{18}:K_1:H_7*E. coli* (extraintestinal), the serotype responsible for newborn meningitis, the H_7 flagellum is involved in infection pathogenesis and the invasion of brain microvascular endothelial cells. Reports indicate that the expression of H_7 flagella by both EHEC and newborn meningitis causing *E. coli* is upregulated after contact with host cells (Haiko and Westerlund-Wikström, 2013).

CONCLUSION

In conclusion, to the best of our knowledge based on our PubMed search, this report is the first publication of an AmpC-producing *E. coli* with a *fliCH*$_7$gene present in healthy chicken. In the current study, the pAmpC-producing *E. coli* isolates that were positive for the *fliCH*$_7$ gene did not belong to the O_{157} serotype and were not STEC strains. However, there are many prevalent *E. coli* strains from several O serotypes with H_7 flagellum and cause extraintestinal infections in both humans and animals. For example, O_1:K_1:H_7 and O_2:K_1:H_7 cause urinary tract infections, septicemia, and neonatal meningitis; and O_{18}:K_1:H_7 serotype causes neonatal meningitis (Delannoy et al., 2017). Therefore, more virulence factors should be investigated in ESBL-/AmpC-producing *E. coli* isolates in the present study to be able to evaluate their pathogenic potential better (for example, for O_{55}:H_7 as an EPEC strain, ETEC, and others).

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CONFLICT OF INTEREST

The author declares no conflict of interest

REFERENCES

Bai J, Paddock ZD, Shi X, Li S, An B, Nagaraja TG (2012) Application of a multiplex PCR to detect the seven major shiga toxin-producing *Escherichia coli* based on genes that code for serogroup-specific O-antigens and major virulence factors in cattle feces. Foodborne Pathog Dis9: 541-548.

Bugarel M, Martin A, Fach P, Beutin L (2011) Virulence gene profiling of enterohemorrhagic (EHEC) and enteropathogenic (EPEC) *Escherichia coli* strains: a basis for molecular risk assessment of typical and atypical EPEC strains. BMC Microbiol11: 142.

Delannoy S, Beutin L, Mariani-Kurkdjian P, Fleiss A, Bonacorsi S, Fach P (2017) The *Escherichia coli* serogroup O1 and O2 lipopolysaccharides are encoded by multiple O-antigen gene clusters.Front Cell Infect Microbiol**7**:30.

Haiko J, Westerlund-Wikström B (2013) The role of the bacterial flagellum in adhesion and virulence. Biology (Basel) 2: 1242-1267.

Huijbers PMC, Graat EAM, Haenen APJ, Van Santen MG, Van Essen-Zandbergen A, Mevius DJ, Van Duijkeren E, Van Hoek AHAM (2014)Extended-spectrum and AmpC β-lactamase-producing *Escherichia coli* in broilers and people living and/or working on broiler farms: prevalence, risk factors and molecular characteristics. J Antimicrob Chemother69:2669-2675.

Osek J (2003) Development of a multiplex PCR approach for the identification of shiga toxin-producing *Escherichia coli* strains and their major virulence factor genes. JAppl Microbiol 95:1217-1225.

Paton AW, Paton JC (2002) Direct detection and characterization of Shiga toxigenic *Escherichia coli* by multiplex PCR for *stx*$_1$, *stx*$_2$, *eae*, *ehxA* and *saa*. J Clin Microbiol 40: 271-274.

Pehlivanoglu F (2017) Existence of Plasmidic AmpC beta lactamase producing *Escherichia coli* isolates in healthy laying hens. Van Vet J 28: 63-67.

Pehlivanoglu F, Turutoglu H, Ozturk D, Yardimci H (2016) Molecular characterisation of *Escherichia coli* isolated from healthy cattle and sheep. Acta Vet-Beograd66: 520-533.

Pehlivanoglu F, Turutoglu H, Ozturk D, Yardimci H (2017) Characterisation of extended-spectrum beta-lactamase producing fecal *Escherichia coli* isolates in laying hens. Ankara UnivVet Fak Derg 64: 301-306.

Posse B, Zutter LD, Heyndrickx M, Herman L (2007) Metabolic and genetic profiling of clinical O157 and non-O157 shiga-toxin-producing *Escherichia coli*. Res Microbiol 158: 591-599.

Reid SD, Selander RK, Whittam TS (1999) Sequence diversity of flagellin (fliC) alleles in pathogenic *Escherichia coli*. J Bacteriol 181:153-160.

Venegas-Vargas C, Henderson S, Khare A, Mosci RE, Lehnert JD, Singh P, Ouellette LM, Norby B, Funk JA, Rust S, BartlettPC, Grooms D, Manning SD (2018)Factors associated with Shiga toxin-producing *Escherichia coli* shedding by dairy and beef cattle. Appl Environ Microbiol 82: 5049-5056.

Wang L, Rothemund D, Curd H, Reeves PR (2003) Species-wide variation in the *Escherichia coli* flagellin (H-Antigen) gene. JBacteriol185: 2936-2943.

WHO (World Health Organisation) (2017) Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. https://www.who.int/medicines/publications/global-priority-list-antibiotic-resistant-bacteria/en/[accessed 15 May 2020]