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\textsubscript{157}:H\textsubscript{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 (*stx*\textsubscript{1}, *stx*\textsubscript{2}, *eae*, *ehxA*, *espP*, *katP* and *saa*) and *E. coli* O\textsubscript{157} (*rfbO*\textsubscript{157}) and H\textsubscript{7} (*fliCH*\textsubscript{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*\textsubscript{7} gene only. When considering the function of the H\textsubscript{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\textsubscript{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*\textsubscript{7}, multidrug resistant
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 O157:H7 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 O157:H7 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 et al., 2017). Information about the source and phylogenetic characteristics of the isolates are presented in Table 1.

The presence of E. coli serotype O157:H7 and STEC/EHEC virulence genes were determined by PCR. PCR was performed for rfbO157, 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 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 gene only. The other genes investigated were also absent in the AmpC-producing E. coli isolates. All the fliCH 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, sulfa methoxazole-trimethoprim, nalidixic acid, enrofloxacin, and tetracycline). All the fliCH7-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 |
|----------------|---------------------|-----------------|---------------------|-------------------|
|                |                     |                 |                     | A0 | A1 | B1 | B2 | B2 | D1 | D2 |
| Cattle         | ESBL                | 8               | 31                  | 2  | 15 | 8  | -  | -  | 3  | 3  |
| Chicken        | ESBL                | 2               | 12                  | -  | -  | 5  | 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 O157:H7 type of E. coli.

| Target gene | Primer sequence (5'------------------3') | Amplicon (bp) | Reference       |
|-------------|----------------------------------------|----------------|-----------------|
| rfbO<sub>157</sub> | F-CAGGTGAAGGTTGGAAATGGTTGTC R-TTGAATTGAGCCACTCAAATAAG | 296 | Bai et al. (2012) |
| fliCH<sub>7</sub> | F-GCTGCAACCGTAAAGTGAT R-GGCAAGCAACGGTTG | 948 | Osek (2003) |
| stx1 | F-TGTCGCACTAGTGAACCTCA R-TGGCCACATGAAGAAGAGA | 655 | Bai et al. (2012) |
| stx2 | F-CCATGACAACCGACAGCTTT | 477 | Bai et al. (2012) |
| eae | F-CATTATGGAACGGAGGAGGT R-ACGGATATCGAAGCTTTT | 375 | Bai et al. (2012) |
| ehxA | F-GCGAGCTAAGCAGCTGTAAT R-CTGGAGAGCTGCACTAATTC | 199 | Bai et al. (2012) |
| espP | F-GATTACAGCACCCATTATGTGTA R-TCAGGAGCTGCACTAATTC | 73 | Posse et al. (2007) |
| katP | F-GAAGTCTATATGCGCGTTGGA R-CTGATTTCCAGGACCGTGAATC | 73 | Posse et al. (2007) |
| saa | F-CTGTGATGAACGGCAGCTTGC R-ATGGAGATCGCCTTGGGAC | 119 | Paton and Paton, (2002) |

Table 3. PCR results for STEC/EHEC virulence genes and O<sub>157</sub>:H<sub>7</sub> type specific genes

| Animal | E. coli isolates n | STEC/EHEC virulence genes and O<sub>157</sub>:H<sub>7</sub> serotype specific genes (n) |
|--------|--------------------|------------------------------------------------------------------|
|        | fliCH<sub>7</sub>  | stx1  | stx2  | eae  | ehxA  | espP  | katP  | saa  |
| Cattle | ESBL  | 31   | -     | -    | -     | -     | -     | -    |
| Chicken| AmpC  | 15   | -     | 6    | -     | -     | -     | -    |
| Sheep  | ESBL  | 12   | -     | -    | -     | -     | -     | -    |
|        | 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<sub>7</sub> gene, coding for the H<sub>7</sub> type flagella. Therefore, our discussion focused on the H<sub>7</sub> 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<sub>7</sub>
type FliC protein were used. Therefore, we could determine the E. coli strain(s) carrying the H7 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, H7 flagella act as adhesins at the initiating step of EHEC infections but did not have any functions during later phases. In O18:K7:H7 E. coli (extraintestinal), the serotype responsible for newborn meningitis, the H7 flagellum is involved in infection pathogenesis and the invasion of brain microvascular endothelial cells. Reports indicate that the expression of H7 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 gene present in healthy chicken. In the current study, the pAmpC-producing E. coli isolates that were positive for the fliCH gene did not belong to the O157 serotype and were not STEC strains. However, there are many prevalent E. coli strains from several O serotypes with H7 flagellum and cause extraintestinal infections in both humans and animals. For example, O3:K1:H7 and O2:K1:H7 cause urinary tract infections, septice mia, and neonatal meningitis; and O18:K7:H7 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 O55:H7, as an EPEC strain, ETEC, and others).

ACKNOWLEDGEMENTS
The primers used in this study were provided from the project 0158-KAYDEP-13 which was initiated by Mehmet Akif Ersoy University Scientific Projects Unit (TURKEY).

CONFLICT OF INTEREST
The author declares no conflict of interest

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