

Characterization of Diarrheagenic *Escherichia coli* Isolated from Fresh Beef, Pork, and Chicken Meat in Korean Markets

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Diarrhea is a major public health concern associated with pathogenic *Escherichia coli* infections. Food-borne pathogenic *E. coli* can lead to large diarrheal outbreaks and hence, there is a need to estimate the frequency of pathogenic *E. coli* load in the various types of meat available in markets. In the present study, we classified and characterized diarrheagenic *E. coli* isolates collected from 399 raw meat samples from retail sources in Korea. Shiga toxin-producing *E. coli* (STEC) were detected in 11 (9.7%) samples, including nine strains (8.0%) in beef and two strains (1.8%) in chicken. The frequency of the detected virulence markers were as follows: *astA*, 28.3%; *escV*, 18.6%; *eaeA*, 17.7%; *ent*, 7.0%; EHEC-*hly*, 4.4%; *stx1*, 3.5%; and *stx2*, 3.5%. We did not observe any typical EPEC, EIEC, or ETEC virulence determinants in any of the samples. The STEC serotype O26 was detected in one sample, but no other serogroups (O91, O103, O128, O157, O145, O111, and O121) were found. Further research is needed to better understand the virulence mechanism of STEC serotypes, their ecology, and prevalence in animals, food, and the environment. These results will help improve risk assessment and predict the sources of food poisoning outbreaks.

Keywords: *Escherichia coli*, enterohemorrhagic *E. coli*, raw meat, multiplex PCR, O serogroup

Introduction

Escherichia coli is the predominant nonpathogenic facultative flora of the human intestine. Some *E. coli* strains, however, have developed the ability to cause diseases of the gastrointestinal, urinary, and central nervous system in even the human hosts with the robust immune system [1]. Diarrheagenic *Escherichia coli* is an important foodborne pathogen that can cause severe diarrheal symptoms. Pathogenic *E. coli* strains are the most common cause of pediatric diarrhea worldwide, is a major public health problem associated with high infant

mortality rates, especially in developing countries [2]. Based on the expression of specific virulence determinants, diarrheagenic *E. coli* can be divided into several pathogroups including shiga toxin-producing *E. coli* (STEC), enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), and enteroaggregative *E. coli* (EAEC) [1, 3].

STEC, also referred to as verotoxin-producing *E. coli* (VTEC), produces either or both classes of bacteriophage-encoded subunit toxins (*stx1* and *stx2*), which are further classified into multiple subtypes. Over 1,000 serotypes of these organisms are abundant in the intestinal tracts of domestic and wild animals, and the environment [2, 4]. In addition to these genes, STEC strains often carry the adherence factor intimin coding *eae* gene along with other virulence genes, such as EHEC-*hly*,

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that code for enterohemolysin [5]. These factors facilitate bacterial adhesion and invasion into intestinal epithelial cells, leading to the formation of severe attaching-effacing (A/E) lesions. The *eae* gene product is the main pathogenic factor in the STEC bacteria, also known as A/E *E. coli*, that is responsible for these lesions [6, 7]. STEC is an important human pathogen in the early 1980s when *E. coli* O157: H7 was linked to outbreaks, and VTEC belonging to several serotypes (O26, O145 etc.) were associated with sporadic cases of the hemolytic uremic syndrome (HUS). Highly pathogenic STEC are now known to cause large outbreaks with a wide spectrum of clinical manifestations that include diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome (HUS). HUS is the leading cause of acute renal failure in children and causes significant morbidity and mortality [8]. Enterohemorrhagic *E. coli* (EHEC) constitutes a subgroup of STEC and can attach to the human intestinal wall to produce A/E lesions. EHEC is considered as the principal cause of HUS [9]. The EHEC O104: H4 outbreak in Germany in the summer of 2011 demonstrated how rapidly an infectious agent could develop into a major health threat for a whole country [10]. ETEC, which is transmitted via the fecal-oral route, causes traveler's diarrhea, and diarrhea in infants through the production of heat-labile and heat-stable enterotoxins [11]. EPEC infection too leads to characteristic A/E lesions. Depending on the presence or absence of the EPEC adherence factor (EAF) plasmid and *bfpB* gene, EPEC is divided into typical (tEPEC) and atypical (aEPEC) subtypes [2]. The expression of the *eaeA* gene is a marker for EPEC infection [12]. The adhesin intimins expressed by tEPEC, aEPEC, and *eae+* STEC, are antigenically heterogeneous, and at least 15 different intimin variants have been recognized in humans and cattle isolates of these bacteria. The most common intimins in human isolates are α (present in some tEPEC serotypes), β (present in some tEPEC, aEPEC, and STEC serotypes), and γ (present in some aEPEC and STEC serotypes including O157: H7) [4]. EIEC closely resembles *Shigella* and causes watery diarrhea and dysentery in severe cases. EIEC is transmitted via contaminated food and water. EAEC strains exhibit a typical adherence pattern in cell cultures, with cells aligning in parallel clusters akin to bricks on a wall [13–16]. EAEC has been linked to sporadic and persistent diarrhea, but

detailed studies of virulence mechanisms have been hindered by the high phenotypic and genotypic diversity of this pathogroup.

E. coli are serotyped based on the profile of their O (somatic), H (flagellar), and K (capsular) surface antigens. Currently, a total of 170 different O antigens, each defining a serogroup, are recognized. A specific combination of O and H antigens defines the “serotype” of an isolate. *E. coli* of specific serogroups are reliably associated with certain clinical syndromes [1]. STEC, particularly O157: H7, are major foodborne pathogens. Six non O157 serogroups, comprising of O26, O103, O111, O145, O121, and O45 are most frequently involved in food poisoning outbreaks [14]. Although several studies have examined the prevalence of EHEC such as *E. coli* O157: H7 and O26 in raw meat, there is limited data on the prevalence of diarrheagenic *E. coli* contamination in raw meats sold in Korea [17].

In this study, we investigated the occurrence of STEC, EPEC, ETEC, EIEC, and EAEC in raw meat samples obtained from various commercial sources in Korea by using multiplex PCR, and analyzed the patterns of virulence gene and *E. coli* O serotypes of identified strains to investigate the contamination level of diarrheagenic *E. coli*. Our results provide a basis for developing strategies to ensure food safety and prevent future outbreaks.

Materials and Methods

Sample collection

Raw meat samples (n = 399) including chicken (n = 133), beef (n = 133), and pork (n = 133) were collected from butcher shops, supermarkets, and open-air markets at various locations in Korea. The samples were placed in sterile polyvinyl bags and transported to the laboratory in a cooler box containing ice blocks. The analysis was performed within 4 h of the sample reaching the laboratory, and the samples were maintained on ice till then.

Strain isolation and identification

E. coli strains in meat samples were identified by the method detailed in the Bacteriological Analytical Manual of the U.S. Food and Drug Administration (<https://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm114664.htm> 2018.06), with some modifications. The 25 g of each meat sample for

enrichment 225 ml of modified tryptic soy broth with novobiocin (Difco, USA) homogenized using a stomacher (400 Circulator; UK) at 260 rpm for 1 min, followed by incubation at 37°C for 18–24 h. The enriched cultures were streaked onto two plates each of endo agar (Merck, Germany) and CHROMagar STEC (CHROMagar, France) and incubated at 37°C for 18 h. At least five typical colonies were selected and analyzed by the gram staining, the catalase test, and biochemical characterization with the Vitek 2 system (bioMérieux, France).

Detection of diarrheagenic *E. coli* by PCR

E. coli isolates were analyzed by multiplex and gradi-

ent PCR (Biometra, Germany) to detect the presence of genes encoding for virulence factors including 1) *uidA*, *invE*, *escV*, *aggR*, or *stx1*; 2) *eaeA*, *elt*, *ipaH*, *stx2*, or *estIb*; and 3) *pic*, *EHEC-hly*, *ent*, or *astA* [9]. The primer pairs used for amplification were purchased from Bioneer (Korea) and are listed in Table 1. PCR was performed in a 20- μ l reaction volume containing Accupower multiplex PCR premix (Bioneer), 1 U Taq polymerase, 250 μ M of each dNTP, 10 mM Tris-HCl, 1.5 mM MgCl₂, and 2–10 pmol of each primer and DNA extracted from single colonies was used as the template. PCR was initiated with a 30 s denaturation step at 98°C, followed by over 35 cycles at 98°C for 30 s, 63°C for 60 s, and 72°C for 90 s,

Table 1. PCR primers used for the detection of virulence genes.

Pathogen	Target gene	Primer sequence	Size (bp)
STEC, aEPEC ¹⁾	<i>eaeA</i>	eae-F: TCAATGCAGTTCGGTTATCAGTT eae-R: GTAAAGTCCGTTACCCCAACCTG	482
	<i>escV</i>	MP3-escV-F: ATTCTGGCTCTCTTCTTTATGGCTG MP3-escV-R: CGTCCCCTTTTACAACTTCATCGC	544
	<i>ent</i>	ent-F: TGGGCTAAAAGAAGACACACTG ent-R: CAAGCATCCTGATTATCTCACC	629
tEPEC ²⁾	<i>bfpB</i>	MP3-bfpB- F: GACACCTATTGCTGAAGTCG MP3-bfpB- R: CCAGAACACCTCCGTTATGC	910
EHEC	<i>EHEC-hly</i>	hlyEHEC-F: TTCTGGGAAACAGTGACGCACATA hlyEHEC-R: TCACCGATCTTCTCATCCCAATG	688
	<i>stx1</i>	MP4-stx1A-F: CGATGTTACGGTTTGTACTGTGACAGC MP4-stx1A-R: AATGCCACGCTTCCCAGAATTG	244
	<i>stx2</i>	MP3-stx2A-F: GTTTTGACCATCTTCGTCTGATTATTGAG MP3-stx2A- R: AGCGTAAGGCTTCTGCTGTGAC	324
EIEC	<i>ipaH</i>	ipaH-F: GAAAACCTCCTGGTCCATCAGG ipaH-R: GCCGGTCAGCCACCCTCTGAGAGTAC	437
	<i>invE</i>	MP2-invE-F: CGATAGATGGCGAGAAATTATATCCCG MP2-invE-R: CGATCAAGAATCCCTAACAGAAGAATCAC	766
EAEC	<i>aggR</i>	MP2-aggR-F: ACGCAGAGTTGCCTGATAAAG MP2-aggR-R: AATACAGAATCGTCAGCATCAGC	400
	<i>pic</i>	MP2-pic-F: AGCCGTTTCCGAGAAAGCC MP2-pic-R: AAATGTCAGTGAACCGACGATTGG	1111
	<i>astA</i>	MP2-astA-F: TGCCATCAACACAGTATATCCG MP2-astA-R: ACGGCTTTGTAGTCCTCCAT	102
ETEC	<i>elt</i>	MP2-LT-F: GAACAGGAGGTTTCTGCGTTAGGTG MP2-LT-R: CTTTCAATGGCTTTTTTTGGGAGTC	655
	<i>estIb</i>	MP2-STI-F: TGTCTTTTTACCTTTGCTC MP2-STI-R: CGGTACAAGCAGGATTACAACAC	171
16s rRNA	<i>uidA</i>	MP2-uidA-F: ATGCCAGTCCAGCGTTTTTGC MP2-uidA-R: AAAGTGTGGGTCAATAATCAGGAAGTG	1487

¹⁾aEPEC, atypical enteropathogenic *E. coli*, ²⁾tEPEC, typical enteropathogenic *E. coli*.

and culminated with a 10-min extension step at 72°C. Amplified products were separated by electrophoresis on a 2% SeaKem agarose gel (Takara Bio, Japan) at 100 V for 25 min. The gel was stained with ethidium bromide (1 µg/ml) solution and amplicons were visualized using UV transillumination system.

Serotyping

The serotypes of STEC isolates were determined by ELISA and PCR; ELISA was performed using *E. coli* group O antisera (Denka Seiken, Japan) and O antigen was detected using the slide agglutination method according to the manufacturer's instructions. PCR was performed using primers targeting either the *rfb* (O-specific polysaccharide), *wzx* (O-unit flippase), or *wbsD* (aminotransferase) gene present in the O-antigen biosynthetic clusters of the eight *E. coli* serogroups (O26, O157, O145, O111, O121, O103, O91, and O128) (Table 2). The reaction volume of 20 µl contained Accupower multiplex PCR premix (Bioneer), 1 U Taq polymerase, 250 µM of each dNTP, 10 mM Tris-HCl, 1.5 mM MgCl₂, and 10 pmol of each primer; DNA extracted from single colonies was used as the template. PCR was initiated with a 5-min denaturation step at 95°C, followed by over 35 cycles at 94°C for 30 s, 55°C for 75 s, and 68°C for 75 s, and culminated with a 5-min extension step at 68°C for

7 min. The amplified products were separated by electrophoresis on a 1.5% SeaKem agarose gel (Takara) at 100 V for 25 min. The gel was stained with ethidium bromide (1 µg/ml) solution and amplicons were visualized using UV transillumination system.

Statistical analysis

The isolation rates of *E. coli* were calculated with monthly positive samples per total meat samples. Seasonal patterns were analyzed by analysis of statistical program used IBM SPSS software (v.20; SPSS Inc., USA) Statistical significance was identified at $p < 0.05$ and $p < 0.001$, separation of the means was accomplished using the probability option (PDIFF, a pairwise t-test).

Results and Discussion

Prevalence of *E. coli* in meats distributed in Korean markets

A total of 113 *E. coli* strains were isolated from 399 samples of three types of raw meat (pork, chicken, and beef), showing a prevalence of 28.3% (range: 7.5–59.4%). Among the three meat types, the chicken was most frequently contaminated with *E. coli* (79/133, 59.4%), followed by beef (24/133, 18.6%) and pork (10/133, 7.5%).

Table 2. PCR primers used for the detection of *E. coli* serogroups.

Pathogen	Target gene (bp)	Primer sequence (5'→3')	Reference
O26	<i>wzx</i> (241)	GCG CTG CAA TTG CTT ATG TA TTT CCC CGC AAT TTA TTC AG	Paddock et al. [14]
O157	<i>rfbE</i> (259)	CGG ACA TCC ATG TGA TAT GG TTG CCT ATG TAC AGC TAA TCC	Valadez et al. [15]
O145	<i>wzx</i> (374)	TGC TCG ACT TTA CCA TCA AC AAC CAA CAC CAT ACA CCT TGT CTT	Valadez et al. [15]
O111	<i>rfb</i> (451)	TAG AGA AAT TAT CAA GTT AGT TCC ATA GTT ATG AAC ATC TTG TTT AGC	Paddock et al. [14]
O121	<i>wzx</i> (587)	TCA TTA GCG GTA GCG AAA GG TTC TGC ATC ACC AGT CCA GA	Paddock et al. [14]
O103	<i>wzx</i> (716)	TTCATCACAAAGTTTCACAAG CGTAATCACCTTGATTTTCT	Paddock et al. [14]
O91	<i>wbsD</i> (940)	GATGAATCAACCTTATCGAG CTGCTTATGTATAGGAATTGG	Paddock et al. [14]
O128	<i>wzx</i> (1353)	TCT TGC TTA TAG CCA GAA TT AAT AAA CCG ACA CCG AAA	Li et al. [25]

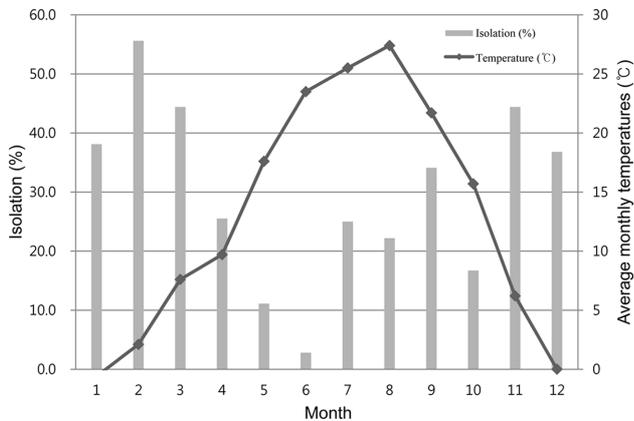


Fig. 1. Month-wise prevalence of *E. coli* isolated from raw meat samples (beef, chicken, pork) in Korea. (Average Monthly temperatures were calculated as the average air temperature at 45 locations in Korea, which are used to calculate the national average according to Korea Meteorological Administration)

Our results were consistent with a previous report from the U.S. (chicken, 38.7%; beef, 19.0%; and pork, 16.3%) [18]. However, an earlier study in Korea found that pork meat was most contaminated with *E. coli* (14.9%) [17]. The monthly prevalence of *E. coli* from January to December ranged from 2.8% to 55.6% (Fig. 1). We

observed a seasonal detection pattern with higher detection rates in February and significant differences ($p > 0.05$) in monthly or seasonal temperatures were determined based on the data from the Korea Meteorological Administration. The average monthly temperatures were calculated as the average air temperature at 45 locations in Korea, which are used to calculate the national average [18].

According to Lee *et al.* [17]: The abundance and shedding of *E. coli* O157: H7 in beef is independent of the season but pork sample a seasonal pattern was observed ($p > 0.05$) with higher rates compared to beef and pork in May, August and September. The prevalence of *E. coli* in four meats (chicken, turkey, pork, beef) varied during the 14 month sampling period, however no seasonality component was observed and these enteric pathogens were found in retail meats in both warm and cold months [19]. Cattle feces samples were isolated *E. coli* O157:H7 every month, the highest monthly prevalence was detected in February and the second highest peak was recorded in August. Overall, no significant differences in prevalence of *E. coli* O157:H7 [20].

The observed differences in prevalence could be due to variations in overall health and immune status of ani-

Table 3. Prevalence of virulence factors among 113 *E. coli* strains isolated from raw meats (beef, chicken, pork).

Pathogen group	Target gene	Number of samples containing virulence marker				
		Beef	Chicken	Pork	Total	%
STEC, aEPEC ¹⁾	<i>eaeA</i>	4	15	1	20	17.7
	<i>escV</i>	5	15	1	21	18.6
	<i>ent</i>	5	2	1	8	7.0
tEPEC ²⁾	<i>bfpB</i>	0	0	0	0	0
STEC	<i>EHEC-hly</i>	5	0	0	5	4.4
	<i>stx1</i>	3	1	0	4	3.5
	<i>stx2</i>	3	1	0	4	3.5
EIEC	<i>ipaH</i>	0	0	0	0	0
	<i>invE</i>	0	0	0	0	0
EAEC	<i>aggR</i>	0	0	0	0	0
	<i>pic</i>	0	0	0	0	0
	<i>astA</i>	3	26	3	32	28.3
ETEC	<i>elt</i>	0	0	0	0	0
	<i>estIa</i>	0	0	0	0	0
	<i>estIb</i>	0	0	0	0	0
<i>E. coli</i>	<i>uidA</i>	21	74	9	104	92

¹⁾aEPEC, atypical enteropathogenic *E. coli*, ²⁾tEPEC, typical enteropathogenic *E. coli*.

mals selected for sampling as well as farm management practices [21]. In addition, meat products that are sold or cut according to the buyer's request may become cross-contaminated by direct transmission of enteric pathogens, which can be avoided by hygienic handling practices.

Presence of virulence gene determinants

Multiplex PCR was used to detect virulence genes harbored by diarrheagenic *E. coli* and the strains were classified as STEC, EPEC, ETEC, EIEC, or EAEC. Virulence genes were detected in 47.8% (54/113) of isolated *E. coli* strains: 49.4% (39/79) in chicken, 45.8% (11/24) in beef, and 40% (4/10) in pork samples. The most common virulence marker was *astA* (28.3%), followed by *escV* (18.6%), *eaeA* (17.7%), and *ent* (7.0%). Interestingly, pathogenic genes were detected at a significantly high frequency in chicken samples (Table 3). Since *E. coli* is a common microorganism present in the intestines of animals, chicken is considered to have the highest detection rate because the whole carcass is produced, processed and distributed [20].

We used the following criteria to determine *E. coli* pathogen groups [1, 22]. For STEC, we evaluated the presence of *stx1* and/or *stx2* and possibly *eaeA*, *escV*, *ent*, and *EHEC-hly*; for EPEC, *eaeA* and possibly *escV* and *bfpB* (with the absence of *bfpB* indicating aEPEC); for ETEC, *elt* and/or *estla* or *estlb*; for EIEC, *invE* and *ipaH*; and for EAEC, *aggR* or a combination of at least two of the three *astA*, *aggR*, and *pic* genes (EAEC marker genes). Of the 54 samples diarrheagenic *E. coli*, EAEC is 25 samples, STEC/EPEC is 22 samples and 7 samples harbored STEC and EAEC markers; and no typical EPEC, EIEC, or ETEC virulence determinants were detected in any of the samples. Several samples contained two or more virulence markers but only aEPEC and STEC were detected (Table 4). Two samples were

negative for *eaeA* but positive for *escV* and *ent*, indicating the presence of EPEC. Although the *astA* gene was detected at a high frequency in this study, we did not detect the *aggR* gene and were, therefore, unable to classify the isolates as EAEC. However, *astA* was simultaneously isolated from six aEPEC and one STEC strain. STEC was detected in 11 (9.7%) samples: nine strains (8.0%) in beef, two (1.8%) in chicken, and none in pork samples (Table 5). These rates are slightly higher than those observed in other countries; STEC was detected in 4% of beef samples in France, 3% of raw beef samples in Australia, and 1.75% of minced beef samples in Switzerland [23]. A higher frequency of STEC has also been reported for beef sampled in Korea [17]. The discrepancies in the contamination level may be due to variations in the meat source and processing environment as well as methodological differences such as the number of samples and sample test period [24, 25]. Contamination can also occur during the processing of slaughtered animals at abattoirs and subsequently amplified by improper storage, handling, or cooking, especially in the case of ground meat [16].

Virulence genes and serotyping of isolated STEC

The genetic profiles of the 11 STEC strains were determined according to the presence of virulence markers such as vero cytotoxin (*stx1* and/or *stx2*) and/or EHEC-*hly*. The serotypes (O26, O157, O145, O111, O121, O103, O91, and O128) of eight STEC strains were determined by PCR. Serotype O26 was detected in one sample of beef, while another could not be typed. O26 was also detected in the *E. coli* O antisera analysis. The serotypes O115 and O1 were detected in beef and chicken samples, respectively, whereas the serotype of other strains could not be determined (Table 5). O26 and O111 serogroups can be pathogenic and are more prominent than O157: H7 in many countries [2]. It is widely accepted that

Table 4. Prevalence of diarrheagenic *E. coli* among 113 *E. coli* strains isolated from raw meats (beef, chicken, pork).

Pathogen group	Samples							
	Beef		Chicken		Pork		Total	
	No.	%	No.	%	No.	%	No.	%
aEPEC ¹⁾	0	0.0	17	15.0	1	0.9	18	15.9
STEC	9	8.0	2	1.8	0	0.0	11	9.8
Total	9	8.0	19	16.8	1	0.9	29	25.7

¹⁾aEPEC, atypical enteropathogenic *E. coli*.

Table 5. O antigen serotyping of isolated Shiga toxin-producing *E. coli* strains.

Sample	Virulence gene	PCR serotype	O antigen serotype
Beef	<i>eaeA/escv/ent/EHEC-hly/uidA</i>		1-4 poly
Beef	<i>escv/ent/EHEC-hly/stx1/uidA</i>	O26	O26
Beef	<i>stx1/uidA</i>		
Beef	<i>stx2/astA/uidA</i>		
Beef	<i>eaeA/eaeA/escv/ent/EHEC-hly/uidA</i>		O115
Beef	<i>eaeA/eaeA/escv/ent/EHEC-hly/stx1/uidA</i>		
Beef	<i>stx2/uidA</i>		
Beef	<i>eaeA/escv/ent/eaeA/EHEC-hly/uidA</i>		
Beef	<i>stx2/astA/uidA</i>		
Chicken	<i>stx2/uidA</i>		
Chicken	<i>stx1/uidA</i>		O1

based on antigen analysis of different serogroups, serotyping can be used to evaluate the pathogenicity of *E. coli*. However, this involves laborious agglutination reactions using antisera raised against O reference strains. In addition, cross-reaction among different serogroups may yield inconsistent results. Earlier, independent studies have suggested that the *stx* gene may not represent a human type of O26, O103, O111, O145, and O157 serotypes [8, 26]. Therefore, strains belonging to the O26, O103, O111, O145, and O157 serotypes may pose the highest risk of human infection. Moreover, there is increasing evidence that different O serogroups of STEC do not harbor the *stx* gene and therefore do not produce shiga toxin. Both, toxigenic (*stx*-positive) and non-toxigenic (*stx*-negative) strains, are likely to coexist within each O serogroup [15]. Non-toxigenic O26 and O157 variants are not of significant concern in terms of food safety/public health. However, occasionally, *stx*-negative *E. coli* have been isolated from human clinical samples. The *stx*-negative, *eae*-positive O26 is likely to be atypical EPEC that is rarely associated with severe outbreaks of human diarrheal disease [27]. The detection of both *stx* and O-serogroup gene sequences in foods indicates the presence of a pathogenic *E. coli* strain. These results warrant further research to better understand the virulence mechanism of STEC, their ecology, and prevalence in animals, and food, and environment.

In summary, our results demonstrate that raw meats sold in Korea have a significant risk of becoming contaminated with pathogenic *E. coli* during processing. Therefore, it is essential not only to wash meat before

preparation to reduce the risk of cross-contaminating the food preparation area but also to properly cook the meat at an appropriate temperature which can effectively destroy pathogenic *E. coli* cells to prevent human infection. These findings underscore the need for vigilance in observing basic hygiene and safety practices during the handling of meat products.

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Conflict of Interest

The authors have no financial conflicts of interest to declare.

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