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NOTE

Occurrence of Pathogenic *Escherichia coli* in Commercially Available Fresh Vegetable Products in Korea

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Abstract Pathogenic *E. coli* is a major foodborne pathogen associated with gastroenteritis worldwide. Fresh vegetable products as well as raw meat and meat products have been recognized as important modes of transmission within the foodborne route. The objective of the present study was to determine the presence of six virulence factors (stx_1, stx_2, *lt*, *st*, *eae*A, and *ial*) in *E. coli* isolated from fresh vegetable products to provide information on risk assessment of pathogenic *E. coli* in Korea. From 416 collected samples, including vegetable salad mix, sprouts, baby leaf vegetables, and unpasteurized fruit and vegetable juices commercially available in Korea, a total of 30 samples were positive for *E. coli* strains, resulting in an overall prevalence of 7.2%. Of the 120 *E. coli* isolates, only one isolate (0.8%), which was obtained from unpasteurized fruit and vegetable juices, was confirmed to possess the *eae*A gene, but lacked *stx* genes. This study showed that some fresh vegetable product samples were contaminated with entero-pathogenic *E. coli*.

Keywords fresh-cut salads · fruit and vegetable juices · pathogenic E. coli · serotype · sprouts

Introduction

Escherichia coli is a ubiquitous commensal bacterium in the intestinal tract of humans and animals and is also implicated in human and animal infectious diseases. Pathogenic *E. coli*, which causes gastroenteritis in humans, is an important foodborne pathogen in different countries including Germany, USA, and Korea (Buchholz et al., 2011; Lee et al., 2012; Luna-Gierke et al., 2014). According to food poisoning statistics, pathogenic strains of *E. coli* accounted for 10.2–16.2% of the total outbreaks in Korea from 2008–2011 (KFDA, 2012).

Pathogenic *E. coli* is generally classified by pathotypes based on virulence traits: enterotoxigenic *E. coli* (ETEC), enteropa thogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), enterohaemorrhagic *E. coli* (EHEC)/Shiga toxin-producing *E. coli* (STEC), and diffuse adherent *E. coli* (DAEC) (Kaper et al., 2004; Xia et al., 2010). STEC has a variety of serotypes. *E. coli* O157:H7 is the most widely recognized STEC serotype, whereas non-O157 STEC serotypes including O26, O103, O111, O113, and O121 are also associated with disease (Acheson, 2000). Most studies on the presence of pathogenic *E. coli* have focused mainly on identifying serotypes of the STEC group. A recent outbreak in several European countries of foodborne illness from pathogenic *E. coli* originating in sprouts highlights the importance of screening for pathogenic *E. coli* in fresh vegetables as well as in raw meat (Buchholz et al., 2011). However, little data is available on the prevalence of pathogenic *E. coli* contamination in fresh vegetable products in Korea.

The objectives of the present study were to identify the prevalence of pathogenic *E. coli* in fresh vegetable products commercially available in Korea, which include vegetable salad mix, sprouts, baby leaf vegetables, and unpasteurized fruit and vegetable juices, and to characterize any pathogenic *E. coli* by virulence groups to provide information for the effective risk assessment of foodborne outbreaks.

Materials and Methods

Collection of samples. A total of 416 samples, which included vegetable salad mix (n =128), sprouts and baby leaf vegetables

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Table 1 Targets and primer sequences used in PCR assays for the identification of virulence genes of pathogenic *E. coli*

Target	Encoding	Primer sequence (5'-3')	Amplicon size (bp)	References
stx_1	Shiga toxin 1	TGTCGCATAGTGGAACCTCA TGCGCACTGAGAAGAAGAGA	655	Bai et al., 2010
stx_2	Shiga toxin 2	CCATGACAACGGACAGCAGTT TGTCGCCAGTTATCTGACATTC	477	Bai et al., 2010
lt	Heat-labile toxin	GCACACGGAGCTCCTCAGTC TCCTTCATCCTTTCAATGGCTTT	218	Vidal et al., 2005
st	heat-stable toxin	TCACCTTTCCCTCAGGATGC ATATTATTAATAGCACCCGG	179	Kimata et al., 2005
*eae*A	intimin	CAGCAGATCATTTTGCCACTCAA CTAAACGGGTATTATCACCAGAAA	574	Ooka et al., 2009
ial	Invasion into eukaryotic cell	GGTATGATGATGATGAGTCCA GGAGGCCAACAATTATTTCC	650	López-Saucedo et al., 2003

(n =176), unpasteurized fruit and vegetable juices (n =109), and herbs and edible flowers (n =3), were collected from retail markets in Korea. Samples were placed on ice in a cooling box after purchasing and were transported to the laboratory within 2 h. They were then immediately stored at 4°C until analysis.

Strain isolation and identification. For the enrichment of the *E. coli* strains, 225 mL of EC broth (Merck, Germany) was added to 25 g of each sample, and then homogenized using a stomacher (®400 Circulator, England) at 230 rpm for 2 min. The mixture was incubated at 37°C for 18 to 24 h. The enriched cultures were streaked onto MacConkey agar (Merck) and incubated at 37°C for 18 h. The plates were inspected for at least five presumptive *E. coli* colonies with typical color and used for further biochemical identification in a VITEK® 2 compact system (Biomerieuk, France).

Polymerase chain reaction (PCR) assays. A total of 120 *E. coli* strains isolated from vegetable salad mix, sprouts, baby leaf vegetables, and unpasteurized fruit and vegetable juices samples were analyzed by multiplex PCR for identification of virulence factors, which included *stx*1 and *stx*2 to identify STEC strains, *eae*A (intimin for *E. coli*-attaching and -effacing) for EPEC, *ial* for EIEC and *lt* (heat-labile toxin) and *st* (heat-stable toxin) for ETEC using a multiple PCR method (López-Saucedo et al., 2003; Kimata et al., 2005; Vidal et al., 2005; Ooka et al., 2009; Bai et al., 2010). PCR amplifications were routinely carried out at a 20 μL reaction volume, which consisted of 1 μL DNA templates: the primer for *E. coli* at 5 pmole/μL; 1 unit of Taq-polymerase, 2.5 mM of dNTP mixture, 10 mM of Tris-HCl (pH 9.0), 30 mM of KCl, and 1.5 mM of $MgCl_2$ (AccuPower™ PCR PreMix, Bioneer, Korea). The targets, primer sequences, amplicon sizes, and cycling conditions for the PCR products are shown in Table 1. The PCR products were electrophoresed on 1.5% agarose gel and stained. The DNA bands were visualized and documented with a GelDoc™ XR+ imaging system (Bio-Rad, USA).

Characterization of pathogenic isolates. Pathogenic *E. coli* isolates, which were identified biochemically were tested by using a latex agglutination test kit for group detection of *E. coli* serogroups O26, O91, O103, O111, O128, and O145 with Dryspot seroscreen® (Oxoid Limited, UK).

Results and Discussion

From the 416 collected samples, a total of 30 samples were positive for *E. coli* strains, resulting in an overall prevalence of 7.2% (Table 2). Among the samples, the unpasteurized fruit and vegetable juices were the most frequently contaminated with *E. coli* (17.4%), followed by sprouts and baby leaf vegetables (4.0%), and vegetable salad mix (3.1%).

Of the 120 *E. coli* strains isolated from the samples, only 1 isolate (0.8%) was confirmed to be pathogenic *E. coli* by the presence of the *eae*A gene (Table 3). The non-O157 serotypes (O26, O91, O103, O111, O128, and O145) as well as serotype O157 have been shown to produce veroxcytotoxin. These non-O157 serotypes were selected due to their most frequent association with verocytotoxin production among non-O157 serogroups (Schroeder et al., 2002). The pathogenic *E. coli* isolate belongs to the investigated serogroups (O26, O91, O103, O111, O128, and O145) but does not possess *stx* genes. The *stx*-negative, *eae*A positive *E. coli* may represent the enteropathogenic *E. coli* (EPEC) pathotype. Previous study on the distribution of virulence genes and their association with serotypes in pathogenic *E. coli* isolates showed that EPEC isolates belong to diverse serotypes including O26 (Cho et al., 2010). In the case of ETEC isolated from diarrheal patients in Korea, the *stx*1 gene was mostly distributed between the O26 and O103 serotypes, whereas the *stx*2 gene was present in the majority of the O157 and O121 serotypes (Cho et al., 2002). The pathogenicity of *E. coli* is determined by virulence factor combinations and not by their serogroups (Schmidt et al., 1993). In the case of STEC isolates, the presence of *eae*A has been suggested to increase their pathogenicity; however, *eae*A negative STEC strains have been implicated in

Table 2 Prevalence of *E. coli* and pathogenic *E. coli* in retail fresh vegetable products

		No. (%) of positive samples		
Sample	Total	*E. coli*	Pathogenic *E. coli*	*E. coli* O157:H7
Sprout and baby leaf vegetables	176	7 (4.0)	0 (0)	0 (0)
Vegetable salad mix	128	4 (3.1)	0 (0)	0 (0)
Unpasteurized fruit and vegetable juices	109	19 (17.4)	1 (0.9)	0 (0)
Herbs and edible flowers	3	0 (0)	0 (0)	0 (0)
Total	416	30 (7.2)	1 (0.2)	0 (0)

Table 3 Distribution of major virulence genes in *E. coli* strains isolated from fresh vegetable products

Virulence gene	No. of positive isolates/No. of total isolates (%)	No. of positive samples/No. of total samples (%)
stx_1	0/120 (0)	0/416 (0)
stx_2	0/120 (0)	0/416 (0)
lt	0/120 (0)	0/416 (0)
st	0/120 (0)	0/416 (0)
*eae*A	1/120 (0.8)	1/416 (0.2)
ial	0/120 (0)	0/416 (0)

sporadic cases of HUS (Bonnet et al., 1998). No other types of *E. coli* were found among any of the *E. coli* isolates. The main type of the pathogens were EPEC (44.7%) and ETEC (34.2%) according to the epidemiological investigation reports of pathogenic *E. coli* outbreak in 2009 (44 reports) and in 2010 (27 reports). EAEC and EHEC were responsible for 10.5 and 9.2%, respectively, of the outbreaks (Lee et al., 2012b).

The identified pathogenic *E. coli* was isolated from unpasteurized fruit and vegetable juices as shown in Table 2. The prevalence of EPEC isolates from retail meat was reported to be 0.9% of 1,275 tested isolates (Xia et al., 2010), which is similar to our data. Most reported data on pathogenic *E. coli* are from animals (mainly ruminants) and meat and milk thereof, because, they are considered to be the main sources of human infections.

Raw vegetables are an important component of the human diet and can be an important source of contamination by foodborne pathogens as well. Outbreaks of STEC infection are increasingly recognized as being associated with fresh vegetable products (EFSA, 2011). Among fresh produces, sprouts and unpasteurized fruit and vegetable juices are recognized to be of public health concern (SCVPH, 2003). These food categories have also been recently reported in the STEC-related Scientific Opinion of EFSA as an important mode of transmission within the foodborne route (EFSA, 2007).

Studies on the prevalence of pathogenic *E. coli* in foodstuffs have mainly focused on meat and meat products in Korea. The prevalence of STEC was investigated in 350 edible beef intestinal samples, and contamination by STEC strains was found in 15 samples (Lee et al., 2012a). From 3,000 samples of retail fresh beef, poultry, and pork meat samples, a total of 39 pathogenic *E. coli* were isolated, and categorized into three virulence groups; namely ETEC (43.6%), EHEC, and EPEC (20.5%) (Lee et al., 2009). Regarding contamination of fresh vegetable products by pathogenic *E. coli*, little information is available in Korea, except for the prevalence of *E. coli* O157:H7 in fresh produces. Current prevalence data showed no *E. coli* O157:H7 contamination on fresh produces in Korea (Kim et al., 2009; Seo et al., 2010).

In conclusion, we investigated the presence of six virulence factors (stx_1, stx_2, *lt*, *st*, *eae*A, and *ial*) in *E. coli* isolated from fresh vegetable products to provide information for risk assessment of pathogenic *E. coli* in Korea. From 416 collected samples, which included commercially available vegetable salad mix, sprouts, baby leaf vegetables, and unpasteurized fruit and vegetable juice products, a total of 30 samples were positive for *E. coli* strains, resulting in an overall prevalence of 7.2%. Of the 120 *E. coli* isolates, only 1 isolate (0.8%), which was obtained from unpasteurized fruit and vegetable juices, was confirmed to possess the *eae*A gene, but lacked *stx* genes. The isolate belongs to the non-O157 Shiga toxin-producing *E. coli* serotypes. To the best of our knowledge, this is the first report on the prevalence of pathogenic *E. coli* other than *E. coli* O157:H7 for commercially available fresh vegetable products including vegetable salad mix, sprouts, baby leaf vegetables, and unpasteurized fruit and vegetable juices in Korea. The data on the occurrence of *eae*A gene positive *E. coli* could have useful information for risk assessment and management.

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