Source tracking of Shiga-like toxin-producing *Escherichia coli* in the fresh vegetable production system of South India

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Abstract

Purpose Numerous outbreaks of foodborne diseases through fresh agricultural produce urge research to assess the source of entry of pathogens to the produce that compromise microbiological safety. In the present investigation, the entry of shiga-like toxin-producing *Escherichia coli* O157:H7 in a fresh vegetable production system was assessed by microbiological and molecular approaches.

Methods Five major vegetables, viz., beetroot, cabbage, carrot, onion, parsley, and potato, being cultivated routinely in the Western Ghats of South India (The Nilgiris), were assessed for the prevalence of *E. coli* O157:H7. The fresh produce, rhizosphere soil, and water resources were sampled and the total coliforms and *E. coli* counts were assessed by plate count method and the O157:H7 by polymerase chain reaction targeting shiga-like toxin gene (*stx1*).

Results The results revealed that all the vegetables collected from the fields had high levels of total coliforms (3 log CFU per g) with high proportions of *E. coli* (1–2 log CFU per g). The prevalence of O157:H7 among the *E. coli* isolates in these vegetables ranged from 0 to 5.8%. However, the prevalence of O157:H7 in rhizosphere soil of these vegetables was relatively high (1.6 to 42.5%). The water used for irrigation and washing the produce (carrot) also showed the presence of O157:H7. The real-time quantitative PCR (qPCR)-based detection of *stx1* revealed that the O157:H7 prevalence in these vegetables and their rhizosphere soil were in higher magnitude than the counts by culturable method. The rhizosphere soil and water samples had higher O157:H7 CFU equivalents than fresh produce.

Conclusions It is evident that the soil as well as the irrigation and process water got contaminated with feces, which are assumed to be the primary source and cause for the entry of O157:H7 to the fresh vegetable. Hence, good agronomical practices and good hygiene post-harvest practices have to be imposed in the vegetable production system to avoid the pathogen entry.

Keywords Fresh produce · *E. coli* O157:H7 · Irrigation water · Rhizosphere · *stx1* gene · Real-time PCR

Introduction

Foodborne diseases, caused by the consumption of food contaminated by bacteria, virus, or chemicals, are the major health burden worldwide with increased morbidity and mortality (Kennedy et al. 2004). A review on foodborne outbreaks in India from 1980 to 2016 showed that important microbial pathogens such as *Staphylococcus aureus*, *Vibrio* sp.,

Salmonella sp., *Escherichia coli*, *Yersinia enterocolitica*, and Norwalk-like virus were responsible for foodborne illnesses (CDC 2018). However, bacterial contamination remains to be the most important source, among which *E. coli* O157:H7 is the leading causative agents with 20% of foodborne-related diseases, next to *Salmonella* sp. (Olsen et al. 2000; Su and Li 2005). *Escherichia coli* O157:H7 is an enterohemorrhagic Shiga toxin-producing foodborne pathogen causing bloody diarrhea, which may progress to the serious complication known as the hemolytic uremic syndrome that leads to severe illness or even death (Karmali 2004).

Fresh vegetables are considered to be an important source of nutritious and healthy diet in many parts of countries. India is the second largest producer of vegetables accounting for 10% of total global production with an annual production of 168.6 million tonnes in an area of 9.6 million ha (NHB 2017). The diverse agro-climatic

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condition, consumers' demand, variety, and availability throughout the year favor the cultivation of almost all types of vegetables. The national nutrition guidelines recommend the average vegetable consumption of 300 g per person per day (NIN 2011). Hence, the demand for fresh vegetables has increased nowadays with concern for healthy lifestyle and balanced diet (Olaimat and Holley 2012). In the past few years, outbreaks caused by consumption of fresh vegetables have increased in developing countries (Söderström et al. 2008; Wendel et al. 2009; Denis et al. 2016; Gutiérrez-Rodríguez et al. 2019). Cattles are the most important reservoir for *E. coli* O157:H7, followed by deer, goats, horses, dogs, and pigs (Smith and Fratamico 2005). However, fresh produce has become the second most important vehicle of transmission associated to *E. coli* O157:H7 outbreak (Julien-Javaux et al. 2019). Therefore, control and prevention of these pathogens using quick and precise detection methods are required for the food industry (O'Grady et al. 2009).

Routine identification and enumeration of *E. coli* in food sample by traditional methods are laborious and time consuming, and sometimes may lead to unpredicted results (Malorny et al. 2003). The PCR-based detection methods have become the valuable and effective tool to detect pathogenic bacteria in different food matrices (Hines 2000) because of their rapid and accurate results. This is mainly essential for fresh-cut vegetables due to their perishable nature. Real-time quantitative PCR (qPCR) is being used as a diagnostic test in the food analysis as an automated high-throughput technique which allows quantification without post-PCR manipulation and with low risk of cross-contamination (Mackay 2004; Mckillip and Drake 2004). Currently, several genes have been used as targets for molecular detection of *E. coli* O157:H7 (Delannoy et al. 2013; Franz et al. 2014) such as Shiga toxin 1 (*stx1*) and Shiga toxin 2 (*stx2*) which are the most critical virulence factors. PCR assays targeting these genes have been developed (Fode-Vaughan et al. 2003). Ibekwe et al. (2004) quantified the natural *E. coli* O157:H7 using multiplex qPCR from phyllosphere and rhizosphere of lettuce. The prevalence of *E. coli* O157:H7 in animal products (Heredia and García 2018) and raw vegetables (Kim et al. 2014; Cardamone et al. 2015; Denis et al. 2016; Alegbeleye et al. 2018) are well documented.

Therefore, the present survey aimed to identify the prevalence and persistence of pathogenic *E. coli* O157:H7 in fresh vegetables and also to explore rapid molecular tools for the effective risk assessment of foodborne outbreaks. We integrated conventional enumeration technique with qPCR, in order to explore the sensitivity, reliability, and quickness of both methods. Therefore, this investigation is of importance as it would contribute to the understanding of the epidemiology of the infection caused by *E. coli* O157:H7 pathogen from agricultural produces.

Materials and methods

Bacterial strain

Escherichia coli O157:H7 strain which was isolated from contaminated irrigation water in Tamil Nadu Agricultural University, Coimbatore, and characterized for the presence of both *stx1* and *stx2* (Shiga-like toxin-producing genes), *eae* (intimin-producing gene) which was designated as O157-TNAU was used as reference strain in this present study (Naganandhini et al. 2015).

Study area and sampling

In the course of this study, five vegetable-growing areas were investigated during March 2018. The chosen areas are located in five neighborhoods in the Kookalthorai region of Ooty, Tamil Nadu, situated at a latitude of 11.49° N and longitude of 76.83° E with an annual average rainfall of 1920.8 mm. The crops in those areas have been produced for more than 20 years. The fresh vegetables, viz., beetroot (*Beta vulgaris*), cabbage (*Brassica oleracea*), carrot (*Daucus carota*), onion (*Allium cepa*), parsley (*Petroselinum crispum*), and potato (*Solanum tuberosum*), and their respective rhizosphere soils along with irrigation water sources were randomly collected for possible isolation of *E. coli* O157:H7. The samples of vegetables were placed in the sterile bags (HiMedia, India) and the rhizosphere soil samples were collected by removing loosely held soil on the roots, into the stomacher bags. The water samples were collected using sterile screw-capped 250-mL glass bottles. All the collected samples were transported under a chilled condition to the Department of Agricultural Microbiology laboratory of the institute and analyses were commenced within 24 h of sample collection.

Physicochemical properties of the rhizosphere soil

The physicochemical properties of rhizosphere soil are presented in Table 1. Soil pH and electrical conductivity (EC) were estimated using a glass electrode with a soil to water ratio of 1:2. The microbial biomass carbon (MBC) was estimated by the fumigation-extraction method (Jenkinson and Ladd 1981). Dehydrogenase (EC 1.1.1.1) was quantified by the reduction of triphenyl tetrazolium chloride to triphenyl formazan (TPF) and expressed in µg of TPF released per g soil per day (Klein et al. 1971).

Bacteriological analysis

For enumerating *E. coli*, Petrifilm EC (3 M™ Petrifilm™ EC, St. Paul, MN, USA) was used by following the manufacturer's instructions. Briefly, 25 g of each vegetable sample was enriched with 225 mL of peptone broth in Baglight® sterile

Table 1 Physicochemical properties of rhizosphere soil collected from farmers' fields

Vegetables	pH	EC (dS/m)	Dehydrogenase (μg TPF released per g soil per day)	Soil biomass carbon ($\mu\text{g/g}$)
Parsley	6.05 (± 0.17) ^{ab}	0.24 (± 0.03) ^{ab}	6.27 (± 0.21) ^a	570.0 (± 75.0) ^{ab}
Potato	5.46 (± 0.03) ^b	0.48 (± 0.01) ^a	5.59 (± 0.74) ^{ab}	675.0 (± 68.7) ^a
Beetroot	6.44 (± 0.09) ^a	0.13 (± 0.02) ^b	4.82 (± 0.57) ^{ab}	405.0 (± 68.7) ^b
Cabbage	5.71 (± 0.22) ^{ab}	0.34 (± 0.14) ^{ab}	1.23 (± 0.17) ^c	585.0 (± 90.0) ^{ab}
Carrot	5.76 (± 0.51) ^{ab}	0.28 (± 0.14) ^{ab}	4.27 (± 0.31) ^b	525.0 (± 79.4) ^{ab}

Values are mean ($\pm$ SE) ($n = 3$) and within each column, values followed by the same letters are not significantly different from each other according to DMRT ($p \leq 0.05$)

bags (Interscience, France) in Class-II Biological safety cabinet (Nuaire, USA). Then the samples were homogenized using a stomacher (BagMixer®, Interscience, France) at 230 min^{-1} for 2 min. For rhizosphere soil, 1 g of respective samples was serially diluted in peptone water and a volume of 1 mL of the appropriate dilutions was placed on the Petrifilm and incubated at 37°C for 24 h. Total coliforms and *E. coli* colonies were enumerated and the results were expressed as log colony-forming units (CFU) per g or mL of sample. On Petrifilm EC plates, total coliforms were calculated by counting blue and red colonies with and without gas production, in which red and blue colonies with gas production were counted as typical *E. coli*.

DNA extraction

An aliquot of 200 μL of suspension from the bagmix was added with 100 μL of Sephadex® G100 (Sigma-Aldrich, St Louis, USA) (10% Sephadex® G100 in 100 mM Tris, 1 mM EDTA, 100 mM NaCl), vortexed for 20 s, and incubated at 95°C for 20 min with intermittent vortexing. After centrifugation at $12000g$ at room temperature for 3 min, an aliquot of 100 μL supernatant was transferred to a new tube and from that 1 μL was used as a template for PCR. Soil DNA was extracted using Fast-DNA™ Spin Kit for Soil (MP Biomedicals, Ohio, US) from 500 mg of soil, following the manufacturer's specifications. All the extracted DNA were quantified spectrophotometrically (Nanodrop 2000, Thermo Scientific, USA), adjusted to 20 ng/ μL working stocks and stored at -20°C .

Colony PCR for detection of O157

A total of 1149 *E. coli* isolates from vegetable, rhizosphere, and farm source were analyzed for detection of Shiga-like toxin-producing *E. coli* (STEC) by PCR targeting the *stx1* gene. The amplification was carried out using *stx1* primers (Forward 5'-CAGTTAATGTGGTGGCGAAGG-3' and reverse 5'-CACCAGACAATGTAACCGCTG-3') (Cebula et al. 1995). The 10 μL PCR reaction mixture contained 1 $\times$ Taq

buffer with 2.5 mM MgCl_2 , 200 μM of each deoxynucleoside 5-triphosphate, 1 μM of each primer, and 2 U of Taq DNA polymerase (HiMedia, India) were adjusted to a total volume with sterile deionized water. Approximately, colonies of 1 mm in diameter were picked up with a sterile toothpick and directly mixed to a PCR tube as a template. PCR amplification was performed in a thermal cycler (Applied Biosystem, USA) with the following conditions: initial denaturation at 95°C for 10 min, followed by 35 cycles of 94°C for 1 min (denaturation), 55°C for 1 min (annealing), 72°C for 1 min (primer extension), and a final extension step of 72°C for 10 min. The PCR products were analyzed by electrophoresis on 1.5% agarose gel stained with ethidium bromide, visualized, and documented using gel document and analysis system (Syngene, UK). The *E. coli* O157-TNAU was used as positive control (Naganandhini et al. 2015).

qPCR analysis

All extracted DNA samples (vegetables, water, and soil) were quantified for STEC by qPCR targeting *stx1*. Quantification was performed in Roche Lightcycler®480II (Roche, Switzerland) in a reaction mixture of 10 μL containing 0.5 μM primer; 5 μL of Lightcycler® 480II SYBR Green I Master mix; and 1 μL of template DNA. PCR was started at 95°C for 10 min, followed by 40 cycles of denaturation at 94°C for 20 s, primer annealing at 65°C for 20 s, and extension at 72°C for 20 s. Fluorescent data collection and data analysis were carried out according to the manufacturer's instructions. C_T , the number of PCR cycles necessary to reach the threshold fluorescence level, was automatically determined for each sample by the software provided with the thermocycler based on the second derivative maximum method. A standard curve was generated by plotting the C_T number against log CFU of O157-TNAU for the three independent replicates, using LightCycler Software v.1 (Roche Applied Science). Amplification efficiency (E) was calculated from the slope of the standard curve using the formula $E (\%) = (10^{-1/\text{slope}} - 1) \times 100$. Amplification specificity was assessed by melting curve analysis of the PCR products

automatically by the thermocycler and the presence of single DNA band of expected size after agarose gel electrophoresis (1.5%). Quantification of gene copies per μL was done by the software provided with the instrument and expressed as CFU equivalents per mL or g of sample.

Statistical analysis

All the data were subjected to statistical analysis with software Microsoft Excel for Windows 2007 add-ins with XLSTAT version 2010.5.05 (XLSTAT 2010). Statistically significant differences between soil samples and *E. coli* strains were analyzed using one-way analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) at 5% significance level.

Results

Physicochemical properties of rhizosphere soil

The physicochemical properties of different rhizosphere soils were significant different among the samples. The pH of soils was slightly acidic (between 5.46 and 6.44) and electrical conductivity varied from 0.13 dS/m (beetroot) to 0.48 dS/m (potato). The dehydrogenase activity and soil microbial biomass carbon were higher in parsley (6.27 $\mu\text{g/g}$) and potato (675.0 $\mu\text{g/g}$) soils respectively as compared to other vegetables (Table 1).

The prevalence of total coliforms and *E. coli*

Among the five fresh vegetables targeted, carrot and cabbage had highest total coliforms (2.90 and 2.86 log CFU/g, respectively) and *E. coli* (2.06 and 1.96 log CFU/g, respectively), followed by beetroot (2.12 and 0.89). The potato had 1.96 log CFU of total coliforms with 1.08 log CFU of *E. coli* (Table 2). Among the five vegetables, parsley had less coliforms with no *E. coli* cells on the surface.

Table 2 The prevalence of total coliforms and *E. coli* in fresh vegetables under field grown conditions

Vegetables	Total coliforms (log CFU/g)	<i>E. coli</i> (log CFU/g)
Parsley	0.44 (± 0.07) ^c	0.00 (± 0.00) ^d
Potato	1.96 (± 0.07) ^b	1.08 (± 0.08) ^b
Beetroot	2.12 (± 0.20) ^b	0.86 (± 0.06) ^c
Cabbage	2.86 (± 0.12) ^a	1.96 (± 0.08) ^a
Carrot	2.90 (± 0.10) ^a	2.06 (± 0.00) ^a

Values are mean ($\pm$ SE) ($n = 3$) and within each column, values followed by the same letters are not significantly different from each other according to DMRT ($p \leq 0.05$)

Compared to vegetables, the rhizosphere soil had a higher population of total coliforms and *E. coli* in all the samples. Among the rhizosphere soils, beetroot, carrot, cabbage, and potato had at par levels of total coliforms (5.36 to 4.27 log CFU/g), but significantly differed levels of *E. coli* counts. Carrot had 4.45 log CFU/g, followed by beetroot (4.34 log CFU), parsley (4.02 log CFU), cabbage (2.99 log CFU), and potato (2.42 log CFU) (Table 3).

Prevalence of *E. coli* O157:H7 in vegetable and rhizosphere soil samples

The colony—PCR-based identification of O157:H7 among the isolated *E. coli* from vegetable— and rhizosphere samples also revealed that rhizosphere soil harbored a higher number of O157:H7 than vegetable samples (Table 4). Among the five vegetables, the percent prevalence of O157:H7 was highest in beetroot (5.8%) followed by cabbage (1.5%). Least proportion of O157:H7 of 0.9% was reported in carrot samples. In parsley, we could not isolate *E. coli*, hence could not perform the PCR screening for O157:H7 identification. However, the rhizosphere soil of parsley had maximum O157:H7 prevalence (42.5%) compared to other vegetables. The rhizosphere soil of carrot had 13.3% O157:H7 followed by beetroot (12.5%), while least ratio of O157:H7 was reported in potato rhizosphere (2.7%) followed by cabbage (1.6%).

The prevalence of total coliforms, *Escherichia coli*, and *Escherichia coli* O157:H7 from the water source

The irrigation water and process water used for carrot washing after harvest had about less than 100 colonies per mL of total coliforms and few colonies of *E. coli* (Table 5). These water samples had 7.7% and 6.1% of O157:H7 respectively. However, the water collected after wash (at two different spots) had threefold increased total coliforms (3.54 and 3.07 log CFU/ml) with an O157:H7 prevalence of 10% and 8.3%, respectively.

Table 3 The prevalence of total coliforms and *E. coli* in rhizosphere soil under field grown conditions

Vegetables	Total coliforms (log CFU/g soil)	<i>E. coli</i> (log CFU/g soil)
Parsley	2.42 (± 1.26) ^b	4.05 (± 0.18) ^a
Potato	4.27 (± 0.11) ^{ab}	2.42 (± 1.21) ^b
Beetroot	5.36 (± 0.34) ^a	4.34 (± 0.20) ^a
Cabbage	4.27 (± 0.49) ^{ab}	2.99 (± 1.50) ^b
Carrot	4.29 (± 0.67) ^{ab}	4.45 (± 0.11) ^a

Values are mean ($\pm$ SE) ($n = 3$) and within each column, values followed by the same letters are not significantly different from each other according to DMRT ($p \leq 0.05$)

Table 4 Presence of *E. coli* O157:H7 in fresh vegetables and rhizosphere soil collected from farmers' field

Vegetables	Vegetable			Rhizosphere soil		
	Total <i>E. coli</i> isolates	Positive for <i>E. coli</i> O157:H7	The prevalence of O157:H7 (%)	Total <i>E. coli</i> isolates	Positive for <i>E. coli</i> O157:H7	The prevalence of O157:H7 (%)
Parsley	—	—	—	40	17	42.5
Potato	104	2	1.9	72	2	2.7
Beetroot	88	5	5.8	80	10	12.5
Cabbage	127	2	1.5	62	1	1.6
Carrot	101	1	0.9	90	12	13.3

Table 5 The prevalence of total coliforms, *E. coli*, and the presence of *E. coli* O157:H7 from the water sources

Water sources	Total coliforms (log CFU/ml)	<i>E. coli</i> (log CFU/ml)	Total <i>E. coli</i> isolates	Positive for <i>E. coli</i> O157:H7	Occurrence of O157:H7 (%)
Carrot washing I	3.54 (± 0.02) ^a	3.38 (± 0.04) ^a	80	8	10.0
Carrot washing II	3.07 (± 0.20) ^b	2.75 (± 0.20) ^a	60	5	8.3
Before wash	1.61 (± 0.04) ^c	0.51 (± 0.02) ^b	49	3	6.1
Irrigation water	1.61 (± 0.11) ^c	0.23 (± 0.05) ^b	26	2	7.7

Values are mean ($\pm$ SE) ($n = 3$) and within each column, values followed by the same letters are not significantly different from each other according to DMRT ($p \leq 0.05$)

Quantification of *E. coli* O157:H7 by qPCR

The qPCR directly quantified the O157:H7 in fresh vegetables collected from the field, rhizosphere soils of those vegetables, and irrigation and process water samples of carrot using the standard curve established after adding DNA from different cell loads (3 to 7 log CFU/ml). The R^2 value of 0.996 was found after regression analysis between CFU per mL and C_T from qPCR (Fig. 1a). The mean amplification efficiencies of qPCR assay for vegetable, rhizosphere soil, and water samples were 92%, 122%, and 119%, respectively. The melt curve analysis of all the samples used in qPCR suggested that there

is only one product in the reaction (Fig. 1b). Among the five, potato and beetroot had relatively high O157:H7 (1.94 and 1.71 log CFU equivalents/g), while the carrot, cabbage, and parsley had at par levels (1.04 to 1.21 log CFU equivalents) (Fig. 2a). However, the rhizosphere soils of these five vegetables had relatively higher CFU equivalents than their respective vegetables (ranged 2.11 to 2.68 log CFU equivalents per g) with insignificant difference among them (Fig. 2b). The irrigation water used for carrot had 4.47 log CFU equivalents, while the wash water before and after also had a range of O157:H7 from 4.29 to 4.65 log CFU equivalents per mL (Fig. 2c).

Fig. 1 **a** qPCR standard curve for *E. coli* O157:H7 generated by plotting the C_T number against CFU/mL of the culture. Error bar indicates the standard error of three independent replicates. **b** Melt curve analysis of qPCR products

Fig. 2 Quantification of Shiga-like toxin-producing *E. coli* by qPCR in fresh vegetables (**a**), rhizosphere soils (**b**), and water used for irrigation and processing (**c**) Means of three replicate values plotted, error bars indicate the standard error. Values followed by the same letter in each panel are not significantly different from each other as determined by DMRT ($p \leq 0.05$)

Discussion

Escherichia coli O157:H7 contamination being an omnipresent foodborne pathogen has caused multitude health issues and mortality due to enterohemorrhagic colitis which was recognized two to three decades ago (Armstrong et al. 1996; Alegbeleye et al. 2018). Despite precautionary measures being strictly followed in developed countries to produce O157-free vegetables and fruits, developing countries like India lack focus in such aspects. In this study, we have surveyed the prevalence of *E. coli* O157:H7 in the list of vegetables preferred for raw consumption that are produced at Nilgiri Mountains and supplied to many parts of south India. Earlier, Sehgal et al. (2008) had reported an increase in *E. coli* O157:H7 population across India during a period of 10 years and pointed out the contribution of animal and water sources in disseminating the same. In the current investigation, we have confirmed that fresh vegetables of the locality were highly prone to *E. coli* O157:H7 from soil and irrigated water. The physicochemical properties of soils measured in the present survey suggested that these soils are conducive for long-term survival of *E. coli* O157:H7 (> 75 days) (Naganandhini et al. 2015).

The World Health Organization (WHO) declared complete destruction of *E. coli* O157:H7 only when entire food is cooked at 70 °C (<http://www.who.int/news-room/fact-sheets/detail/e-coli>). The issue remains unresolved when vegetables are freshly consumed as salads and fast foods (carrot, cabbage, onion etc.) and used for garnishing (coriander, parsley etc.) (Abdul-Raouf et al. 1993; Sharapov et al. 2016). Our study was focused on such vegetables and total coliforms and *E. coli* loads were higher in rhizosphere soil compared to vegetables collected from the same field (Tables 2 and 3 of the present study). This indicated that soil acting as a source of contamination may be at the time of conventional harvest and piling up of vegetables at the field before transport. Most interestingly, there was no prevalence of *E. coli* in parsley herb while its rhizosphere soil had higher count ($4.05 \pm 0.18 \log \text{CFU g}^{-1}$). Several factors can affect bacterial survival on fruits and vegetables at the pre-harvest stage, including UV exposure (Muela et al. 2000; Steele et al. 2005), temperature (Han and Linton 2004), produce surface drying (Steele et al. 2005), and antimicrobial activity (Hashem and Saleh 1999). The antimicrobial activity associated with the high ferrous sequestering nature of stem extracts of parsley inhibited *E. coli* (Wong and Kitts 2006; Petrolini et al. 2013). Moreover, soil dehydrogenase activity directly correlated with *E. coli* load since both were recorded highest in parsley and lesser in cabbage rhizosphere soil (Tables 1 and 4). In accordance with total coliforms and *E. coli*, percentage occurrence of O157:H7 was significantly higher in rhizosphere soil (1.6 to 42.5%) compared to that of vegetables collected (0.9 to 5.8%) (Table 5) which confirmed generic *E. coli* to be the indicator for the O157:

H7 occurrence. Further, the persistence of *E. coli* O157:H7 in agricultural soils poses a major threat to fresh vegetables cultivated in that locality (Naganandhini et al. 2015). The factors such as application of pre-matured compost (Mukherjee et al. 2007), contaminated manure and irrigation water (Solomon et al. 2002; Islam et al. 2004), inorganic fertilizer amendment (Franz et al. 2008), and non-sanitized wash water (Gil et al. 2009) were so far reported to contribute for abundance of O157:H7 in vegetables. In the present study, we have quantified the presence of *E. coli* O157:H7 in both irrigation and wash water used in that locality. The count of O157:H7 was detected higher in wash water used for carrot processing and the count gets even higher after wash (Table 5). This may lead to more hazardous effects as the same water is being reused for washing the next slot leading to a heavy buildup of inoculum in carrot samples after processing. The carrot being the highly preferred raw vegetable in that locality has also emerged as a vehicle of O157:H7 transmission to humans due to unhygienic conventional cultivation and processing.

qPCR is considered as a method of choice for the detection and quantification of microorganisms (Postollec et al. 2011). qPCR of vegetable samples is superior for detecting pathogenic *E. coli* over conventional cultural and serological techniques (Liming and Bhagwat 2004). The predominance of STEC-type carrying *stx1* sequences in some vegetables, such as carrot, parsley, which are consumed raw, represents a serious risk to public health. The presence of *eaeA* in STEC seropathotypes O157 and O157:H7 was reported in cattle, cattle carcasses, and environmental samples in Istanbul by multiplex PCR (Yilmaz et al. 2006). In the same way, *stx1* was used as a marker to detect STEC using qPCR in the current work and highly virulent O157:H7 were identified in all the samples considered (carrot beetroot, potato, cabbage, and parsley).

To conclude, the study described the source of entry of *E. coli* O157:H7 contamination in a fresh vegetable production system of South India. The results indicate that most of the fresh vegetables growing in this locality can mediate O157:H7-based foodborne illness through soil and water contamination. Scientific attention regarding sanitized wash water and hygienic cultivation practices in such localities, which act as a hub for fresh vegetable supply, would help prevent loss of human life due to STEC infection. Hence, providing knowledge-based agricultural production system to the farmers and developing strategies to minimize the foodborne illness in farm to fork approach are necessary in order to ensure the safety of fresh produce delivering to the consumers.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Research involving human participants and/or animals N/A

Informed consent N/A

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