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ORIGINAL ARTICLE

Molecular sequence typing reveals genotypic diversity among *Escherichia coli* isolates recovered from a cantaloupe packinghouse in Northwestern Mexico

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Significance and Impact of the Study: Packinghouses can be considered as a potential source of microbial contamination. Using multilocus sequence typing, this study identified a genotypic and phylogenetic diverse set of *Escherichia coli* isolates recovered from the surfaces of cantaloupes, workers' hands and processing equipment at a cantaloupe packinghouse. A total of 61% of the sequence types identified were novel, and a distinct sequence type, ST-827, was significantly associated with worker's hands, sampled during the final postwash operational stages in the packinghouse. These findings serve as a baseline to identify potential sources of microbial contamination at distinct operational stages in a cantaloupe packinghouse.

Keywords
cantaloupe, diversity, *Escherichia coli*, food safety, genotyping, postharvest.

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Abstract

The increase in the consumption of fresh produce has correlated with a rise in the number of reported foodborne illnesses. To identify potential risk factors associated with postharvest practices, the present study employed multilocus sequence typing (MLST) for the genotypic classification of *Escherichia coli* isolates recovered from three sources sampled at seven operational stages in a cantaloupe packinghouse in Northwestern Mexico. The MLST analysis results indicated that the *E. coli* isolates were classified into 18 different sequence types (ST), and 11 of these STs were found to be novel. ST-171 was the predominant type and was found in 19% (7/36) of the recovered isolates. Interestingly, the novel ST-827 was found to be significantly associated with isolates recovered from workers' hands, sampled during final postwash stages. Further phylogenetic analyses to examine the relatedness of the STs revealed genetic heterogeneity. Fourteen of the identified STs were assigned to known clonal groups, while the remaining four novel STs were distinct and did not cluster with any clonal group. The present study has provided the first evidence indicating that several sources from distinct operational stages in a cantaloupe packinghouse may contribute to a genotypic and phylogenetic diverse set of *E. coli* isolates.

Introduction

Over the past decades, the increased consumption of fresh produce has contributed to a demand for year-round availability (Sivapalasingam *et al.* 2004; Callejón *et al.* 2015). Import trade agreements to provide products that are out of season in the United States have helped increase the supply. In a 20-year period, between 1980 and 2001, both fresh fruits and vegetables imports increased by 155 and 265% respectively (Clemens 2015). In particular, Mexico has become the largest supplier of fresh fruits and vegetables to the United States (Huang 2013; Clemens 2015). Moreover, recent government initiatives in the United States have been launched to

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increase fresh produce consumption (USDHH and USDA 2015). The trend of increased consumption of fruits and vegetables has been followed by a rise in the number of reported outbreaks (Sivapalasingam *et al.* 2004; Callejón *et al.* 2015; FDA 2016).

Cantaloupes have been frequently implicated in produce-associated outbreaks (Sivapalasingam *et al.* 2004; Walsh *et al.* 2014). Cantaloupes imported from Mexico were implicated in outbreaks from 2000 to 2002 resulting in import restrictions to the United States (Walsh *et al.* 2014; FDA 2016). More recently, cantaloupes, grown in the United States, were associated with a deadly *Listeria monocytogenes* outbreak in 2011 as well as a *Salmonella enterica* outbreak in 2012 (Walsh *et al.* 2014; FDA 2016). When compared to other melon varieties, cantaloupes have a rough and netted surface, which may enable the adherent bacteria to resist removal by washing treatments (Parnell *et al.* 2005; Walsh *et al.* 2014).

Packinghouses are considered as potential sources of microbial contamination (Walsh *et al.* 2014; Heredia *et al.* 2016). As a result of postharvest processing, fresh produce in a packinghouse come into contact with multiple surfaces and sources. Moreover, inefficient sanitation of handlers and processing equipment as well as improper disinfection of wash water can contribute to harbour micro-organisms and contaminate the fresh produce (Walsh *et al.* 2014; Heredia *et al.* 2016). Previous reports identified risk factors associated with sources of contamination in cantaloupe packinghouses, located in southern states in the United States and eastern and western states in Mexico (Castillo *et al.* 2004; Johnston *et al.* 2006; Alvarado-Casillas *et al.* 2007; Ailes *et al.* 2008; Heredia *et al.* 2016). However, studies on potential sources of contamination in cantaloupe packinghouses from other agricultural states in Northwestern Mexico are lacking. One of these agricultural states is Sonora, which is known for having an active cantaloupe industry for exportation (Stout *et al.* 2004; FDA 2016). In particular, no research evidence is available on the genotypic classification of recovered *Escherichia coli* isolates from different sources and operational stages in a cantaloupe packinghouse in Sonora.

To identify potential risk factors associated with postharvest handling and processing practices, the present study employed a sequence-based method, multilocus sequence typing (MLST), for the genotypic characterization of *E. coli* isolates recovered from various sources and operational stages in a cantaloupe packinghouse. The results revealed that the surfaces of cantaloupes, workers' hands and processing equipment from distinct operational stages in the packinghouse are all potential routes of a genotypic and phylogenetic diverse set of *E. coli* isolates. These findings have set a precedent for the evaluation of potential sources of contamination and could aid in the development of preventive measures to reduce bacterial contamination of cantaloupes in packinghouses in Northwestern Mexico.

Results and discussion

Typing of recovered *E. coli* isolates

Given that postharvest handling and processing practices can be a potential source of microbial contamination (Walsh *et al.* 2014), the present study conducted a genotypic characterization of a subset of *E. coli* isolates recovered from multiple sources at a cantaloupe packinghouse. After sampling the surfaces of the cantaloupe, worker's hands and packinghouse equipment, a total of 36 *E. coli* isolates were recovered at seven distinct stages that were part of the packinghouse operations (Fig. 1). Moreover, a multiplex PCR assay revealed that the isolates did not harbour virulence genes specific for common types of diarrheagenic *E. coli* (data not shown). Cantaloupes were the predominant source of the examined isolates, and this source accounted for 69% (25/36) of the *E. coli* isolates recovered from most operational stages (Table 1 and Fig. 2a). The later operational stages, stages 5 through 7, yielded all of the *E. coli* isolates from worker's hands, representing 16% (6/36) of the recovered isolates. Sampling the packinghouse equipment surfaces resulted in the recovery of 14% (5/36) of the isolates from stages 2, 5 and 7. No isolates were recovered from operational stage 3. The number of isolates recovered from each stage of processing continued to increase as fruit progressed along the packinghouse (Fig. 2a). In particular, the final operational stages 5 through 7, postwash stages that require produce handling (Fig. 1), accounted for 81% (29/36) of the recovered isolates (Table 1 and Fig. 2a). Our observations were in agreement with previous reports documenting an increased microbiological contamination of cantaloupe at the later postwash stages in the packinghouse operation (Castillo *et al.* 2004; Ailes *et al.* 2008; Akins *et al.* 2008).

To better characterize the recovered *E. coli* isolates, MLST was performed to analyse the genetic relatedness among isolates (Table 1). MLST was selected as the typing method for this study since it is a technique that has enabled the discrimination of isolates not distinguishable by other methods such as pulse-field gel electrophoresis (Qi *et al.* 2004). The results indicated that the isolates were classified into 18 different sequence types (ST), and 11 of these STs were found to be novel (Table 1). A total of 17% (6/36) of the isolates possessed novel combinations with new MLST alleles, and 31% (11/36) of the isolates were found to have novel combinations of known MLST alleles. Altogether, a total of 47% (17/36) of the isolates were found to have a novel ST. ST-171 was the

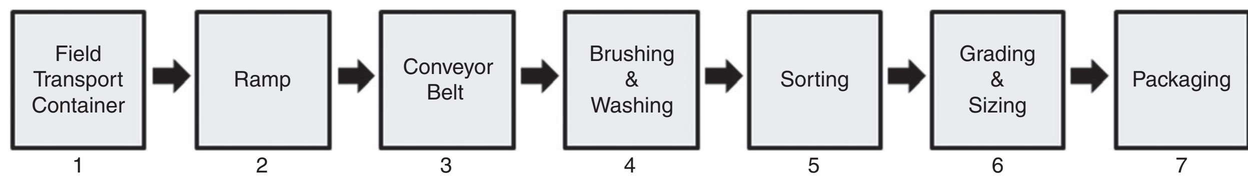

Figure 1 Operational stages in a cantaloupe packinghouse. The cantaloupe packinghouse, examined in the present study, was located in the state of Sonora, Mexico. The distinct operational stages in the packinghouse are ordered by sequence of processing from first (left) to last (right).

Table 1 *Escherichia coli* isolates examined in this study and their corresponding multilocus sequence typing (MLST) results

				Allele number							
Source	Stage*	Isolate	ST†	*aspC*	*clpX*	*fadD*	*icdA*	*lysP*	*mdh*	*uidA*	CG‡
Cantaloupe melon	1	FB2	171	3	3	1	1	1	1	1	23
		FB1	824§	7	5	64	4	115	5	1	60
	4	FC8	134	5	6	13	25	1	8	1	30
		FC4	171	3	3	1	1	1	1	1	23
		FC3	243	5	5	13	2	1	13	23	31
		FC7	826§	4	6	2	2	1	13	231	ND
	5	FS10	171	3	3	1	1	1	1	1	23
		FS8	254	7	5	77	4	1	5	95	43
		FS4	823§	3	115	194	177	1	103	145	ND
		FS6	825§	4	173	193	4	1	13	230	ND
		FS7	825§	4	173	193	4	1	13	230	ND
		FS3	829§	32	5	61	4	1	12	5	59
	6	FCL5	88	32	12	61	12	1	12	12	13
		FCL10	153	5	5	2	4	1	13	63	40
		FCL6	171	3	3	1	1	1	1	1	23
		FCL2	243	5	5	13	2	1	13	23	31
		FCL3	243	5	5	13	2	1	13	23	31
		FCL4	254	7	5	77	4	1	5	95	43
		FCL7	823§	3	115	194	177	1	103	145	ND
	7	FE10	153	5	5	2	4	1	13	63	40
		FE5	171	3	3	1	1	1	1	1	23
		FE4	243	5	5	13	2	1	13	23	31
		FE9	254	7	5	77	4	1	5	95	43
		FE3	830§	5	5	13	2	2	13	23	31
		FE6	831§	7	5	77	4	1	13	95	43
Packinghouse equipment	2	SR2	461	5	5	2	30	1	8	1	41
	5	SS1	827§	5	6	2	4	1	5	1	68
	7	SE2	171	3	3	1	1	1	1	1	23
		SE3	833§	5	4	2	4	1	5	1	80
		SE1	834§	4	5	2	2	1	13	1	ND
Worker's hands	5	MS1	827§	5	6	2	4	1	5	1	68
		MS2	827§	5	6	2	4	1	5	1	68
	6	MCL1	827§	5	6	2	4	1	5	1	68
		MCL2	827§	5	6	2	4	1	5	1	68
		MCL3	832§	7	5	2	4	1	5	1	60
	7	ME2	171	3	3	1	1	1	1	1	23
Human	NA	EDL933	66	1	1	4	3	2	4	4	11
		K-12	171	3	3	1	1	1	1	1	23

*The number refers to the packinghouse operational stages as described in Fig. 1. NA refers to not applicable.
†ST refers to sequence type.
‡Clonal groups (CG) assigned as described in the Materials and methods. ND refers to not determined.
§Novel ST not previously described in the *Ec*MLST database.

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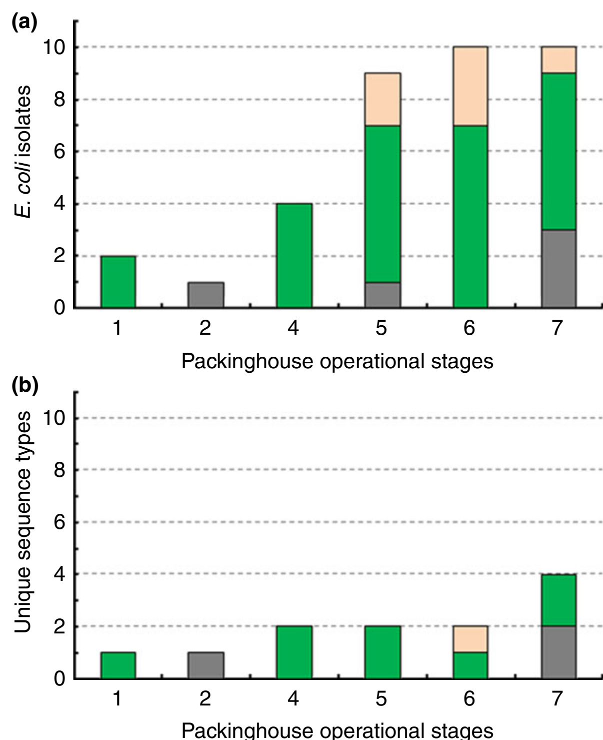

Figure 2 Total count of (a) recovered *Escherichia coli* isolates and (b) unique sequence types (STs) identified from distinct operational stages and sources at a cantaloupe packinghouse. The packinghouse operational stages along the *x*-axis are described in Fig. 1. The *Escherichia coli* isolates were recovered by sampling the surfaces of packinghouse equipment (grey bars), worker's hands (beige bars) and cantaloupes (green bars). [Colour figure can be viewed at wileyonlinelibrary.com]

predominant type and was found in 19% (7/36) of the isolates recovered from all sources in five out of the seven operational stages in the cantaloupe packinghouse. Several reports have previously documented ST-171 to be common among *E. coli* isolates, recovered from geographically widespread locations and diverse sources, including humans, animals and the environment (Steinsland *et al.* 2010; Contreras *et al.* 2011; Feng *et al.* 2012).

Furthermore, the MLST analysis also revealed some genetic heterogeneity in the recovered isolates since 33% (12/36) of the examined isolates possessed a ST only found in a single isolate (Table 1). We then examined whether the isolates recovered per source at a particular operational stage had unique STs (Fig. 2b). The results indicated that the surfaces of cantaloupes led to the recovery of isolates with unique STs in four out of the seven operational stages. By contrast, distinct STs were identified in isolates from worker's hands (ST-832) at only operational stage 6 or from equipment surfaces at stages 2 and 7 (ST-833 and ST-834) (Fig. 2b and Table 1). Our analyses also revealed a significant correlation between an isolate genotype and a particular source. A total of 80% (4/5) of the isolates harbouring the novel ST-827 were recovered from workers' hands, and this observation was found to be statistically significant (Fisher's exact test, $P = 0{\cdot}0012$). No other significant associations were found between ST and either source or operational stage of the packinghouse.

Clustering and phylogenetic analyses

To examine the genetic relatedness of the identified ST in the recovered *E. coli* isolates, a phylogenetic tree was constructed (Fig. 3). For the phylogenetic analysis, closely related STs were assigned into a clonal group (CG). Given that 61% of the identified STs in the present study were novel (Table 1), this finding prompted the inclusion of known STs for the construction of the dendrogram and enabled the assignment of the novel ST to a CG, based on a high bootstrap value (Contreras *et al.* 2011; Isiko *et al.* 2015). Fourteen of the identified STs in the recovered isolates were assigned to 11 CGs (Fig. 3). Seven of the novel STs (ST-824, ST-827, ST-829, ST-830, ST-831, ST-832 and ST-833) were found to cluster with known CGs. By contrast, the remaining four novel STs (ST-823, ST-825, ST-826 and ST-834) were distinct and did not cluster with previously identified CGs (Fig. 3). Interestingly, the dendrogram showed that the cantaloupe isolates belonging to ST-243 and ST-254 clustered with ST-145 and ST-221 respectively. Previous studies have identified ST-145 and ST-221 in strains of Shiga toxin-producing *E. coli* (Isiko *et al.* 2015), and this observation suggests that these cantaloupe isolates may be closely related to STs identified in *E. coli* strains harbouring *stx*-encoding mobile elements.

The genomic analysis was then expanded to examine the genetic variability of the recovered isolates. A low allelic variability with values below 10 sites per locus was observed in MLST loci *aspC* and *lysP* (Table S1). A higher variability of over 20 variables sites was observed for the other loci, as previously reported in a distinct geographical population of *E. coli* isolates from clinical samples (Contreras *et al.* 2011). The pairwise homoplasy index test (Φ_w statistic) revealed a statistically significant indication of recombination in the alleles of *fadD*, *icdA* and *uidA* (Table S1). In summary, the present study provided the first evidence indicating that several sources from distinct operational stages in a cantaloupe packinghouse contribute to a genotypic and phylogenetic diverse set of *E. coli* isolates.

Materials and methods

Bacterial reference strains and growth conditions

Escherichia coli O157:H7 strain EDL-933 (DEC 4f) was obtained from *E. coli* Reference Center, The Pennsylvania State University, PA (Reid *et al.* 1999). *Escherichia coli*

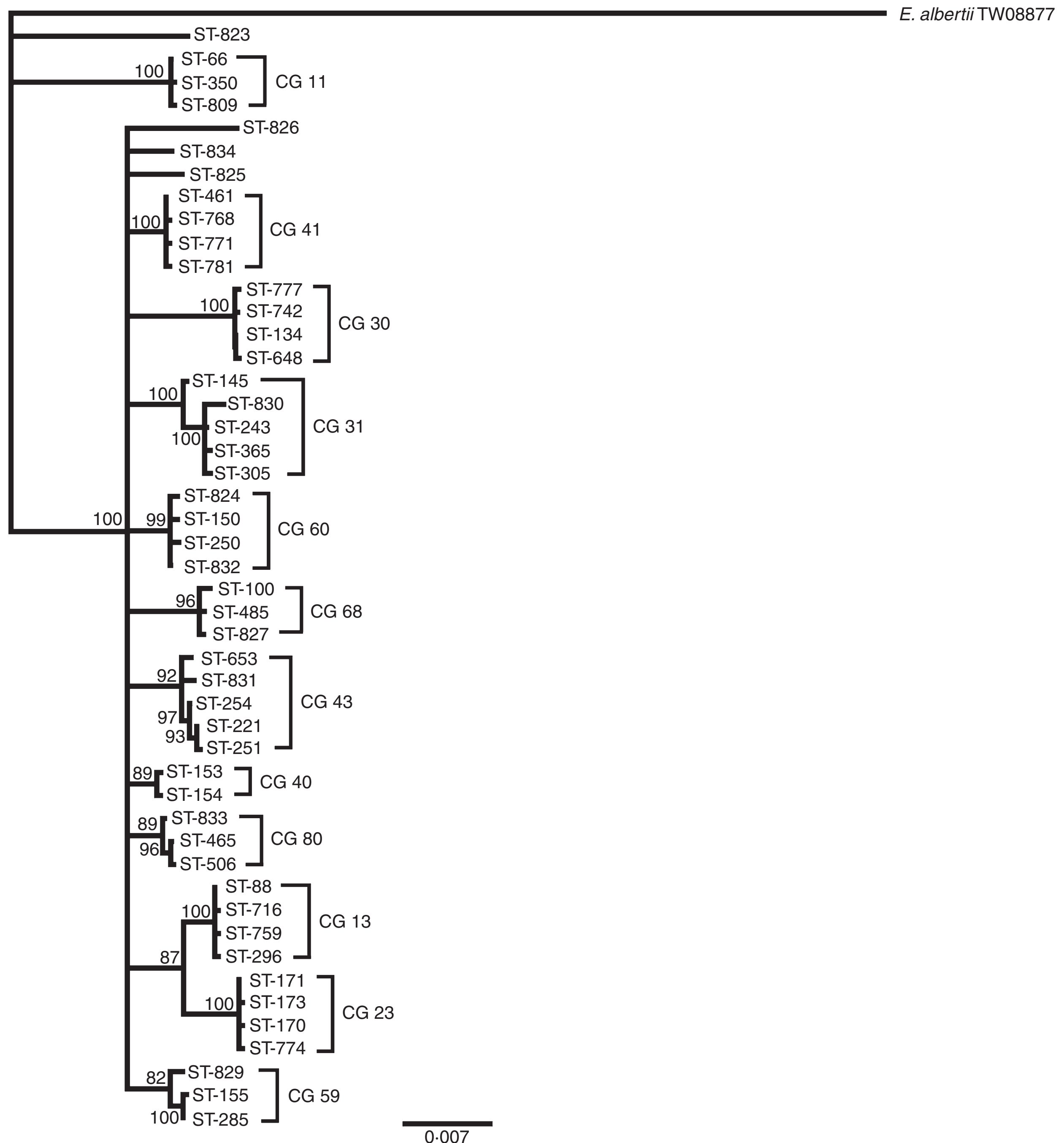

Figure 3 Phylogenetic tree of sequence types (STs) identified in the recovered *Escherichia coli* isolates. The phylogenetic tree was generated by using the neighbour-joining and the Jukes-Cantor distance methods. Sequence types of isolates recovered from the cantaloupe packinghouse are shown in bold. The assignments of STs to clonal groups (CGs) are indicated with square brackets. Bootstrap values, shown at internal notes, were generated from 5000 replications. The scale bar corresponds to 0·7 nucleotide substitutions per 100 nucleotides.

strain K12 (ATCC 29425) was obtained from the American Type Culture Collection (Manassas, VA). Bacterial cultures were propagated in Difco, Luria-Bertani (LB) agar (BD Diagnostics, Sparks, MD) or grown under aerobic conditions with constant shaking (200 rev min^{-1}) at 37°C in Difco LB broth (BD Diagnostics).

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Sample collection, processing and microbiological analyses

Sample collection was conducted during a 1-month period at a cantaloupe packinghouse, located in the state of Sonora, Mexico. The presence of *E. coli* was determined by using the membrane filtration technique (Soto-Beltrán *et al.* 2015) from randomly selected samples of 70 cantaloupe (10 samples per stage), 70 samples of sorting and packaging equipment surfaces (10 samples per stage) and 40 samples from hands of workers (10 samples from stages 2 and 5–7) at seven distinct operational stages in the packinghouse (Fig. 1). Cantaloupe surfaces were peeled, placed in a sterile Ziploc bag (S. C. Johnson, Mexico City, Mexico) containing 225 ml of Difco-buffered peptone water (BD Diagnostics) and mixed vigorously for 1 min (Castillo *et al.* 2004). Furthermore, the sampling from the equipment surfaces in the packinghouse (receiving ramp, conveyor belts, brushing, sorting, grading/sizing and packaging) was performed by swabbing an area of approximately 2500 cm^2 using a sterile 3·8 × 7·6 cm Whirl-Pack Hydrated Speci-Sponge (Nasco, Fort Atkinson, WI), moistened with 5 ml 2% Difco-buffered peptone water (BD Diagnostics). The workers' hands (pickers, sorters and packers) were sampled by rubbing their hands with a sterile swab moistened with Difco-buffered peptone water (BD Diagnostics) for 40 s (Castillo *et al.* 2004). Each sponge, used for sampling equipment and the workers' hands, was mixed with 95 ml of buffered peptone water in a Ziploc bag (S. C. Johnson) and then homogenized manually (Soto-Beltrán *et al.* 2015). For the recovery of *E. coli* from all samples, 100 ml of the buffered peptone water was filtered through a sterile GN-6 Metricel 0·45 μm pore size mixed cellulose ester membrane (Pall Corporation, Port Washington, NY) (Alonso *et al.* 1999; Soto-Beltrán *et al.* 2015). Filters were then placed on indicator solid medium, CHROMagar ECC (CHROMagar Microbiology, Paris, France) (Alonso *et al.* 1999). *Escherichia coli* colonies with a typical blue colour appearance were selected and grown subsequently in Difco tryptic soy agar (BD Diagnostics) for 24 h. Presumptive colonies were further examined by performing a multiplex PCR assay to identify genes (*eae, bfpA, ial, elt, est, stx1* and *stx2*) specific for some common types of diarrheagenic *E. coli* identified in Mexico, as in previous studies (López-Saucedo *et al.* 2003). All PCR amplifications were performed using reaction mixtures and cycling conditions as recently described (Soto-Beltrán *et al.* 2015).

Multilocus sequence typing of *E. coli* isolates

The *E. coli* isolates in Table 1 were typed by using primer sets for PCR amplification of internal portions of seven housekeeping genes (*aspC*, *clpX*, *fadD*, *icdA*, *lysP*, *mdh*, *uidA*) (Qi *et al.* 2004), following PCR protocols as described in the *E. coli* MLST (*Ec*MLST) database system (http://www.shigatox.net/new/tools/ecmlst.html) (Qi *et al.* 2004). Cycle sequencing reactions were performed on a Dyad Peltier Thermal Cycler (Bio-Rad, Hercules, CA) using the ABI PRISM BigDye terminator cycle sequencing kit (ver. 3.1; Applied Biosystems, Foster City, CA), and extension products were purified by using BigDye XTerminator Purification Kit (Applied Biosystems) following the manufacturer's specifications. DNA sequencing was performed on an ABI PRISM 3130X Genetic Analyser using POP-7 polymer and the ABI PRISM Data Collection software ver. 3.0. DNA chromatograms were visualized and assembled using LASERGENE software ver. 8.0 (DNAstar, Inc., Madison, WI). All allelic sequences were queried against the *Ec*MLST database system to assign numbers to alleles already present in the database (Table 1), and the combination of alleles from the seven housekeeping genes was used to determine the ST of each isolate (Qi *et al.* 2004). All novel allelic sequences and STs not found in the *Ec*MLST database system were assigned new numbers by the database curator. STs that were highly related were assigned into CG by the *Ec*MLST database system. Novel STs were assigned to a CG based on a high level of bootstrap value (>80%) to determine the relatedness of the novel ST to others in a CG (Isiko *et al.* 2015).

Phylogenetic and statistical analyses

To determine genetic relationships, sequences from the seven housekeeping genes were aligned and concatenated, and a phylogenetic tree was constructed with GENEIOUS 6.1.8 software (Biomatters, Ltd., Aukland, New Zealand) by using the neighbour-joining and the Jukes-Cantor distance methods. The phylogenetic tree was rooted with sequences from *Escherichia albertii* strain TW08877, and bootstrap values were generated from 5000 replicates (Isiko *et al.* 2015). Further analysis of the genotypic diversity was determined by calculating the *P*-value for the pairwise homoplasy index (Φ_w statistic) test, using the SPLITSTREE4 computational application (Tübingen University, Tübingen, Germany) for distinguishing recurrent gene mutations from recombination (Bruen *et al.* 2006). Significance for the pairwise homoplasy index corresponded to a $P \leq 0{\cdot}05$ (Bruen *et al.* 2006). Correlations between the STs of the isolates and their distinct sources or packinghouse operational stages were analysed by performing a two-tailed Fisher's exact test using the R-Statistical Software (ver. 3.0.1; R Foundation for Statistical Computing, Vienna, Austria). A $P \leq 0{\cdot}01$ was considered to be statistically significant.

Acknowledgements

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Conflict of Interest

No conflict of interest declared.

References

Ailes, E.C., Leon, J.S., Jaykus, L.A., Johnston, L.M., Clayton, H.A., Blanding, S., Kleinbaum, D.G., Backer, L.C. *et al.* (2008) Microbial concentrations on fresh produce are affected by postharvest processing, importation, and season. *J Food Prot* **71**, 2389–2397.

Akins, E.D., Harrison, M.A. and Hurst, W. (2008) Washing practices on the microflora on Georgia-grown cantaloupes. *J Food Prot* **71**, 46–51.

Alonso, J.L., Soriano, A., Carbajo, O., Amoros, I. and Garelick, H. (1999) Comparison and recovery of *Escherichia coli* and thermotolerant coliforms in water with a chromogenic medium incubated at 41 and 44.5 degrees C. *Appl Environ Microbiol* **65**, 3746–3749.

Alvarado-Casillas, S., Ibarra-Sánchez, S., Rodríguez-García, O., Martínez-Gonzáles, N. and Castillo, A. (2007) Comparison of rinsing and sanitizing procedures for reducing bacterial pathogens on fresh cantaloupes and bell peppers. *J Food Prot* **70**, 655–660.

Bruen, T.C., Philippe, H. and Bryant, D. (2006) A simple and robust statistical test for detecting the presence of recombination. *Genetics* **172**, 2665–2681.

Callejón, R.M., Rodríguez-Naranjo, M.I., Ubeda, C., Hornedo-Ortega, R., García-Parrilla, M.C. and Troncoso, A.M. (2015) Reported foodborne outbreaks due to fresh produce in the United States and European Union: trends and causes. *Foodborne Pathog Dis* **12**, 32–38.

Castillo, A., Mercado, I., Lucia, L.M., Martínez-Ruiz, Y., Ponce de León, J., Murano, E.A. and Acuff, G.R. (2004) *Salmonella* contamination during production of cantaloupe: a binational study. *J Food Prot* **67**, 713–720.

Clemens, R.L. (2015) The Expanding U.S. Market for Fresh Produce. *Iowa Ag Review*. **10**, 8–9.

Contreras, C.A., Ochoa, T.J., Ruiz, J., Lacher, D.W., Rivera, F.P., Saenz, Y., Chea-Woo, E., Zavaleta, N. *et al.* (2011) Phylogenetic relationships of Shiga toxin-producing *Escherichia coli* isolated from Peruvian children. *J Med Microbiol* **60**, 639–646.

FDA, U.S. Food and Drug Administration (2016) *Import Alert 22-01: Detention Without Physical Examination of Cantaloupes From Mexico*. Silver Springs, MD: U.S. Food and Drug Administration.

Feng, P.C., Keys, C., Lacher, D.W., Beutin, L., Bentancor, A., Heuvelink, A., Afset, J.E., Rumi, V. *et al.* (2012) Clonal relations of atypical enteropathogenic *Escherichia coli* O157:H16 strains isolated from various sources from several countries. *FEMS Microbiol Lett* **337**, 126–131.

Heredia, N., Caballero, C., Cárdenas, C., Molina, K., García, R., Solís, L., Burrowes, V., Bartz, F.E. *et al.* (2016) Microbial indicator profiling of fresh produce and environmental samples from farms and packing facilities in northern Mexico. *J Food Prot* **79**, 1197–1209.

Huang, S.W.(2013) *Imports Contribute to Year-Round Fresh Fruit Availability, FTS-356-01*. Washington, DC: U.S. Department of Agriculture, Economic Research Service, U.S. Government Printing Office.

Isiko, J., Khaitsa, M. and Bergholz, T.M. (2015) Novel sequence types of non-O157 Shiga toxin-producing *Escherichia coli* isolated from cattle. *Lett Appl Microbiol* **60**, 552–557.

Johnston, L.M., Jaykus, L.A., Moll, D., Anciso, J., Mora, B. and Moe, C.L. (2006) A field study of the microbiological quality of fresh produce of domestic and Mexican origin. *Int J Food Microbiol* **112**, 83–95.

López-Saucedo, C., Cerna, J.F., Villegas-Sepúlveda, N., Thompson, R., Velázquez, F.R., Torres, J., Tarr, P.I. and Estrada-García, T. (2003) Single multiplex polymerase chain reaction to detect diverse loci associated with diarrheagenic *Escherichia coli*. *Emerg Infect Dis* **9**, 127–131.

Parnell, T.L., Harris, L.J. and Suslow, T.V. (2005) Reducing *Salmonella* on cantaloupes and honeydew melons using wash practices applicable to postharvest handling, foodservice, and consumer preparation. *Int J Food Microbiol* **99**, 59–70.

Qi, W., Lacher, D.W., Bumbaugh, A.C., Hyma, K.E., Ouellette, L.M., Large, T.M., Tarr, C.L. and Whittam, T.S. (2004) *Ec*MLST: an online database for multi locus sequence typing of pathogenic *Escherichia coli*. In *Proceedings - 2004 IEEE Computational Systems Bioinformatics Conference, CSB 2004*. pp.520–521.

Reid, S.D., Betting, D.J. and Whittam, T.S. (1999) Molecular detection and identification of intimin alleles in pathogenic *Escherichia coli* by multiplex PCR. *J Clin Microbiol* **37**, 2719–2722.

Sivapalasingam, S., Friedman, C.R., Cohen, L. and Tauxe, R.V. (2004) Fresh produce: a growing cause of outbreaks of foodborne illness in the United States, 1973 through 1997. *J Food Prot* **67**, 2342–2353.

Soto-Beltrán, M., Campo, N.C., Campos-Sauceda, J., Avena-Bustillos, R. and Chaidez, C. (2015) Prevalence of *Salmonella, Escherichia coli* and coliforms on bell peppers from the field to packing house process. *Afr J Microbiol Res* **9**, 718–724.

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Steinsland, H., Lacher, D.W., Sommerfelt, H. and Whittam, T.S. (2010) Ancestral lineages of human enterotoxigenic *Escherichia coli*. *J Clin Microbiol* **48**, 2916–2924.

Stout, J., Huang, S., Calvin, L., Lucier, G., Perez, A. and Pollack, S. (2004) NAFTA trade in fruits and vegetables. In *Global Trade Patterns in Fruits and Vegetables*. ed. Huang, S. pp. 39–51. Washington, DC: U.S. Department of Agriculture, Economic Research Service, U.S. Government Printing Office.

USDHH and USDA, U.S. Department of Health and Human Services and U.S. Department of Agriculture (2015) *In 2015–2020 Dietary Guidelines for Americans*, 8th ed. Washington, DC: U.S. Government Printing Office.

Walsh, K.A., Bennett, S.D., Mahovic, M. and Gould, L.H. (2014) Outbreaks associated with cantaloupe, watermelon, and honeydew in the United States, 1973–2011. *Foodborne Path Dis* **11**, 945–952.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Sequence variations in loci used for multilocus sequence typing (MLST).