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Genetic Relatedness Among *Escherichia coli* Pathotypes Isolated from Food Products for Human Consumption in Cartagena, Colombia

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Abstract

Foodborne pathogens are a leading cause of mild-to-severe gastrointestinal illnesses worldwide. *Escherichia coli* pathotypes have been known to cause gastrointestinal illnesses in children less than 5 years old in Colombia. However, insufficient information is available on the prevalence of *E. coli* contamination of food products and the kind of *E. coli* food product reservoirs. The two objectives of this study were designed to address this issue. The first objective was to ascertain coliform, *E. coli*, and pathogenic *E. coli* contamination of food products readily available for human consumption in Cartagena, Colombia. The second objective was to evaluate the relationship between pathogenic *E. coli* isolated from food products and those isolated from cases of diarrhea in children. Food product samples consisting of pasteurized milk, unpasteurized fruit juice, ground beef, cheese, and vegetables were obtained at four retail stores. The food samples were cultured in liquid media and tested for the presence of coliforms and *E. coli*. *E. coli* isolates were tested by polymerase chain reaction for the presence of pathogenic *E. coli*. Coliforms, *E. coli,* and *E. coli* intestinal pathotypes contamination were detected in 88.4%, 53%, and 2.1% of food product samples, respectively. Ground beef and cheese were the only food samples contaminated with *E. coli* intestinal pathotypes including enteropathogenic (EPEC), Shiga toxin–producing (STEC), and enterotoxigenic *E. coli* (ETEC). Closed multilocus sequencing typing relationships between diarrheagenic *E. coli* isolates from food products and from individuals with diarrhea suggest that food products readily available at public markets in Cartagena can transmit ETEC and possibly EPEC and STEC. We demonstrated that a high proportion of food products for human consumption available at public markets in Cartagena are contaminated with coliforms, *E. coli*, and *E. coli* intestinal pathogens. Furthermore, food products containing *E. coli* intestinal pathogens may be involved in the transmission of foodborne illnesses among children in Cartagena, Colombia.

Introduction

Diarrheagenic *Escherichia coli* are well-known foodborne pathogens worldwide. They are classified into six distinct pathogenic categories based on their mechanisms of pathogenesis: enterotoxigenic (ETEC), Shiga toxin–producing/enterohemorrhagic (STEC/EHEC), enteropathogenic (EPEC), enteroinvasive (EIEC), enteroaggregative (EAEC), and diffusely adherent (DAEC) *E. coli* (Kaper *et al*., 2004). Shiga toxin–producing *E. coli* (STEC) O157:H7 strains are highly virulent foodborne pathogens associated with outbreaks of bloody diarrhea, and hemolytic uremic syndrome (HUS) in the United States, Europe, and other countries around the world (Tarr *et al*., 2005). O157 STEC infections have been estimated to cause 73,000 illnesses in 2005 in the United States, resulting in more than 2000 hospitalizations and 60 deaths (Gould *et al*., 2013a).

Although clinical studies have indicated the importance of *E. coli* pathotypes in childhood diarrhea in Colombia, studies on food product contamination by *E. coli* pathogens in Colombia are limited (Gómez-Duarte *et al*., 2010; Rúgeles *et al*., 2010). A few studies report that meats and vegetables at retail were contaminated with two pathogenic *E. coli* strains, STEC and EAEC (Mattar and Vásquez, 1998; Rúgeles *et al*., 2010). However, no study has evaluated the genetic relatedness between diarrheagenic *E. coli* derived from food products and those isolated from children with diarrhea.

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A recent case–control study reported a high prevalence of *E. coli* diseases in Cartagena among children less than 5 year old (Gomez-Duarte *et al*., 2013). It led us to ask whether foodborne and/or waterborne pathogens are playing a role in the epidemiology of *E. coli* intestinal illnesses. The objective of this study was to identify the most frequently contaminated food products for human consumption and the most frequent *E. coli* intestinal pathogens. More importantly, we evaluated whether diarrheagenic *E. coli* from food products share similarities with previously reported diarrheagenic *E. coli* from children with diarrhea.

Materials and Methods

Food product collection

Food products were purchased at four main retail stores (stores 1–4) from 2012 to 2013. The stores were located in four different neighborhoods within the city of Cartagena, Colombia. Nineteen samples corresponding to five consumable products (cheese, ground beef, vegetables, pasteurized milk, and unpasteurized juice) were collected at each store for a total of 380 samples. Vegetables collected were carrots, spinach, celery, and/or lettuce. Vegetables collected were fresh, and displayed in open air. None of the vegetables used were prepacked, precooked, or frozen. Solid food samples were collected in aseptic plastic bags. Pasteurized milk was collected in the original container. Unpasteurized juice samples were collected in sterile bottles. All samples were transported at 8°C to the microbiology laboratory at the University of Cartagena for further processing.

Processing of food products

Solid and liquid food product samples were evaluated for bacterial growth, presence of coliforms, and *E. coli*. Ten grams of solid food product samples were added to 90 mL peptone buffered water (EMD Millipore, Billerica, MA) and homogenized in a sterile blender for 1–2 min at low speed. Liquid samples were not mixed with peptone buffered water. Two hundred milliliters of lactose broth (EMD Millipore) was inoculated with 1 mL of homogenized solid food or liquid food sample. The suspension was incubated at 37°C for 24 h with shaking. Samples with no bacterial growth on lactose broth were discarded. Samples positive for bacterial growth with no gas production were presumed to contain Gram-positive organisms and were also discarded. Food samples positive for bacterial growth with gas production in lactose broth were assumed to contain Gram-negative organisms. Lactose fermenter bacterial suspensions were subsequently cultured on Fluorocult medium (Fluorocult® LMX broth; Thomas Scientific, Swedesboro, NJ) to determine total coliforms and *E. coli*. One-hundred milliliters of Fluorocult broth was inoculated with 1 mL of lactose broth bacterial suspension (gas-producer) and incubated for 24 h at 37°C. Fluorocult medium bacterial suspensions that emitted fluorescence under ultraviolet light were considered positive for *E. coli*. An aliquot of the suspension was plated on McConkey agar for isolation of individual colonies. Individual bacterial isolates were confirmed as *E. coli* by the presence of lactose-fermenting colonies on McConkey agar, metallic green colonies on eosin methylene blue (EMB) agar, positive testing for indol, β-glucuronidase, methyl red, and negative testing for citrate. Up to two *E. coli* isolates per food sample were stored in Luria broth (LB) supplemented with 20% glycerol at −80°C for further testing.

DNA amplification by polymerase chain reaction (PCR)

Multilocus sequence typing (MLST). *E. coli* clinical isolates were analyzed by MLST as described online (http://mlst.warwick.ac.uk/mlst/). Internal fragments from seven housekeeping genes were amplified by PCR and DNA sequenced as described before (Wirth *et al*., 2006). Sequence editing was conducted with DNADynamo software (Blue Tractor Software Ltd., North Wales, UK). Seven genes sequences for each strain were concatenated to generate a 3423-bp aligned DNA sequence, and concatamers were aligned using ClustalW software (available online at: http://www.phylogeny.fr/version2_cgi/simple_phylogeny.cgi) (Dereeper *et al*., 2008, 2010). Phylogenetic trees from *E. coli* MLST concatamers were conducted by the bootstrapping procedure. *E. coli* control MLSTs included STEC, EPEC, and ETEC strains reported in the http://mlst.warwick.ac.uk/mlst/ database and previously isolated from human cases of diarrhea. Control *E. coli* ancestral phylogenetic groups sequences were also obtained from the same database. MLST-based clonal groups were defined as a group of more than one *E. coli* strain MLST sequence that do not seem to share ancestral origin with other *E. coli* and that have at least two strains with identical MLST DNA sequence.

DNA amplification by PCR assays were performed as described earlier with slight modifications (Gómez-Duarte *et al*., 2010). In brief, individual bacterial isolates were cultured overnight on 2 mL LB at 37°C with shaking. Bacterial cultures were centrifuged, pellet resuspended in Tris-EDTA buffer (10 mM Tris, pH 8.0, 0.1 mM EDTA in distilled water), and boiled for 5 min at 95°C. Bacterial suspensions were centrifuged and the crude genomic DNA within the supernatant was transferred to a fresh tube and used as DNA template for PCR reactions. Multiplex PCR (mPCR) for detection of *E. coli* pathotypes was performed as described earlier (Gannon *et al*., 1997; Gómez-Duarte *et al*., 2010) with the following modifications. Two reactions were run for each sample using two different sets of primers. Each 25-μL reaction mixture contained 23 μL of Platinum Blue PCR SuperMix (Invitrogen, Carlsbad, CA), 1 μL of primer mix (Mix1 or Mix2) (Supplementary Table S1; Supplementary Data are available online at www.liebertpub.com/fpd), and 1 μL of template genomic DNA. Primer Mix1 (58°C annealing temperature) was prepared for amplification of *eae*/*bfpA*, *stx1/stx 2*, and *aggR* for detection of EPEC, STEC, and EAEC pathotypes, respectively. Primer Mix2 (56°C annealing temperature) was prepared for amplification of ST/LTb, *virF/ipaH*, and *daaE* for detection of ETEC, EIEC, and DAEC, respectively. Both PCR mixes were denatured at 94°C for 5 min, followed by 40 cycles of 92°C for 30-s denaturation, annealing for 30 s (at 56°C for PCR mix1 and 58°C for 30 s for PCR mix2), and extension for 30 s at 72°C. PCR products were separated on 3% (wt/vol) agarose gel in Tris acetate EDTA buffer (pH 8.2), stained with ethidium bromide (10 μg/mL), visualized, and recorded under ultraviolet light. Diarrheagenic *E. coli* reference strains were used as positive controls: EPEC E2348/69 (*eae* +, *bfpA* +), STEC O157:H7 2060-004 (*eae*, *stx1*, *stx2*), EIEC EC-12 (*ipaH, virF*), DAEC C1845 (*daaE*), and EAEC JM221 (*aggR*)

(Gómez-Duarte *et al*., 2009). The *E. coli* DH5 α strain (negative for all virulence genes) was used as a negative control. Each pathotype identified by mPCR was confirmed by single PCR amplification of specific gene targets. This mPCR assay has been validated in prior studies, and differential amplification of DNA did not affect assay sensitivity or specificity (Gómez-Duarte *et al*., 2009, 2010).

Serotyping

O and H typing of diarrheagenic *E. coli* isolates were performed at Penn State *E. coli* Reference Center (Pennsylvania State University, University Park, PA). O serotyping was conducted using antisera generated against *E. coli* serogroups designated O1-O187 with the exceptions of O31, O47, O67, O72, O94, and O122, as these are not yet designated. H typing was performed by PCR–restriction fragment length polymorphism of *fliC* flagellar gene responsible for H types.

Antimicrobial susceptibility testing

BBL™ Prompt™ Inoculation System (Becton Dickinson & Co., Franklin Lakes, NJ) was used to prepare standardized bacterial suspensions for the Bauer-Kirby disc-diffusion antimicrobial susceptibility test. In brief, bacterial isolates were grown overnight on LB agar plates at 37°C. Five individual colonies were harvested to create a bacterial suspension following the manufacturer recommendations. Muller-Hinton agar plates were inoculated with the BBL Prompt Innoculation system bacterial suspension. Antimicrobial susceptibility to 12 different antibiotics was tested using BD BBL™ Sensi-Disc™ Susceptibility Test Discs methods (Becton, Dickinson and Company). Strain activity was tested against cefazolin (CZ), ceftriaxone (CTX); ampicillin (A), amoxicillin/clavulanic acid (AC); ceftazidime (CZ), cefuroxime (CX), cefepime (CP), ciprofloxacin (CIP), gentamicin (GM), meropenem (MEM), trimethoprim/sulfamethoxazole (STX), and piperacillin/tazobactam (TZP). The zones of bacterial growth inhibition were interpreted according to the guidelines of the Clinical and Laboratory Standards Institute (Qin *et al*., 2008). The *E. coli* ATCC 29522 strain was used as the negative control (susceptible to all antibiotics). *Klebsiella pneumoniae* ATCC 700603D-5 strain was used as the positive control (resistant to all β-lactam antibiotics).

Statistical analysis

Fisher exact test and one-way analysis of variance were used to determine statistical significances of the differences among food product samples or among retail stores. All statistical calculations were done using IBM SPSS Statistics for Windows, Version 22. For all calculations, the p value for statistical significance was set at < 0.01.

Results

Identification of total coliforms and E. coli *isolates from food products*

Three hundred eighty food samples collected from four different retail stores in Cartagena, Colombia were assayed to detect total coliforms and *E. coli* contamination (Supplementary Tables S2 and S3). Coliform was found in 334 of the 380 samples. Cheese, ground beef, unpasteurized juice and vegetables were the most frequently contaminated products, with 297 (97.7%) samples positive out of 304 (Supplementary Table S2). In contrast, pasteurized milk was the least frequently contaminated food product, with only 37 (48.7%) samples positive out of a total of 76. The lower frequency of pasteurized milk contamination was statistically significant ($p < 0.01$) when compared to the remaining products. The numbers of coliforms-contaminated products per retail store ranged from 80 to 89 of 95 samples each. The difference in numbers was not statistically significant, suggesting a similar frequency of coliform contamination across retail stores in Cartagena.

Food products contaminated with *E. coli* were detected in 201 (52.8%) of 380 samples (Table 1). Cheese and ground beef were the most frequently contaminated samples at 96% and 100%, respectively (Supplementary Table S3). Pasteurized milk, unpasteurized juice, and vegetable samples were less frequently contaminated. Pasteurized milk was the least frequently contaminated product, affecting only 6.6% of the samples. The difference in the frequencies of *E. coli* contamination between pasteurized milk and ground beef or cheese was statistically significant ($p < 0.01$). Similarly, the difference in *E. coli* contamination between unpasteurized juice or vegetables when compared to either ground beef or cheese was statistically significant ($p < 0.01$). As with the coliform, the frequencies of *E. coli*–contaminated products across the retail stores were similar to each other, and the differences were not statistically significant.

Diarrheagenic E. coli *isolates present in ground beef and cheese samples*

Diarrheagenic *E. coli*, defined as those isolates carrying virulence genes unique to EPEC, STEC, and ETEC, were identified in 8 (2.0%) of the 380 food samples (Table 1). The frequency of contamination of samples with diarrheagenic *E. coli* in all the samples that were contaminated with *E. coli* was 4.0%. Diarrheagenic *E. coli* was found in 9.2% of the ground beef samples. From eight diarrheagenic *E. coli* strains identified, seven were detected in ground beef and one in a cheese sample. Four ground beef samples were contaminated with STEC non-O157, three samples with ETEC, and one sample with EPEC. The diarrheagenic *E. coli*–contaminated cheese sample was positive for EPEC (Table 2).

Diarrheagenic E. coli *isolates from food-product-shared serotypes and MLST with human diarrheagenic* E. coli *isolates*

To compare the diarrheagenic *E. coli* isolates from food products and the previously reported diarrheagenic *E. coli* from children with diarrhea, we performed O:H serotyping and MLST. All *E. coli* pathotype strains identified from food products were serotyped. A total of 4 O serogroups were identified, including O8, O25, O126, and O128. In one of the strains, no O serogroup was found. A total of 6 H serogroups were identified including H10, H12, H16, H19, H28, and H+. All 4 STEC strains belong to a single O8 serogroup, 2 of them belong to H19 serogroup, and the remaining 2 to H28 serogroup (Table 2).

MLST analysis was performed on all diarrheagenic *E. coli* isolates from food samples to study their genetic diversity. We identified five previously reported sequence types and two novel sequence types among these *E. coli* isolates (Table 2).

Table 1. Identification of *Escherichia coli* from Food Products

	Negative E. coli		*Positive* E. coli							
			Pathotypes		*No pathotypes*		*Total* E. coli		*Total*	
Sample	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*
Cheese	3	4	1	1	72	95	73	96	76	100
Pasteurized milk	71	93	0	0	5	7	5	7	76	100
Unpasteurized juice	55	72	0	0	21	28	21	28	76	100
Ground beef	0	0	7	9	69	91	76	100	76	100
Vegetable	50	66	0	0	26	34	26	34	76	100
Total	179		8		193		201		380	

The 2 new sequence types ST4495 and ST4496 have been submitted to the MLST database. They were present in three STEC isolates. These strains also expressed O8 serogroup (Table 2). A phylogenetic tree constructed based on the MLST concatamer sequence alignments revealed a close relationship among *E. coli* pathotypes sharing identical serotype. STEC strains CCtF203.1 and CCtF203.2 share identical O8:H28 serotype and MLST sequence type. Similarly, STEC strains CCtF047 and CCtF054 share an identical O8:H19 serotype and grouped closely in the MLST phylogenetic tree (Fig. 1). All identified *E. coli* pathotypes from food products clustered with the strains that belong to the ancestral groups B1 and A. No *E. coli* isolate clustered with the ancestral groups B2 or D.

MLST analysis revealed that all ETEC strains from food products grouped with the previously reported Colombian human ETEC strains (Fig. 1). The ETEC CCtF197 isolate had an MLST sequence type identical to the human ETEC clinical isolates COCt123 and COCt90cc and to the ancestral *E. coli* ECOR9 strain (Fig. 2). These data suggest that the ETEC isolates and the *E. coli* ECOR9 strain belong to the sequence type ST10 and to the ancestral *E. coli* group A (Figs. 1 and 2).

Low antimicrobial resistance identified among E. coli *isolated from food products*

Resistance to ampicillin, amoxicillin/clavulanic acid, and trimethoprim/sulfamethoxazole was detected in 7/8 (87.5%), 6/8 (75.0%), and 1/8 (12.5%) diarrheagenic *E. coli* isolates, respectively. Six of eight diarrheagenic *E. coli* strains were resistant to more than one antibiotic. One EPEC strain recovered from a cheese sample was resistant to all of the three antibiotics. Twenty-six *E. coli* food product isolates, 8 diarrheagenic (Table 2) and 18 nondiarrheagenic, were evaluated for antimicrobial susceptibility. Similar to diarrheagenic *E. coli*, nondiarrheagenic strains were predominantly resistant to ampicillin and amoxicillin/clavulanic acid. They were sensitive to the remaining antibiotics (data not shown).

Discussion

Foodborne illnesses are associated with high morbidity and mortality among humans in both industrialized and developing countries. Bacterial pathogens are the primary etiological agents in foodborne illnesses (Khabbaz *et al*., 2014). This study reports bacterial contamination in a large proportion of food products available at retail markets in Cartagena, Colombia. Detection of coliforms, *E. coli*, and diarrheagenic *E. coli* pathogens among food product samples is an indication of fecal contamination. Furthermore, this level of contamination places the local community, especially children, at a higher risk of foodborne illnesses.

E. coli contamination of ground beef, cheese, and vegetables is an evidence of fecal contamination that may occur at any stage in the food chain, including production, harvesting, storage, packaging, or purchasing. Contamination of vegetables frequently occurs after irrigation using untreated sewage water (Castro-Rosas *et al*., 2012). Ground beef contamination generally occurs during slaughtering and processing, while cheese contamination may occur during manufacturing and processing (Vernozy-Rozand *et al*., 2005; Gould *et al*., 2011). No difference in the total numbers of

Table 2. Genotype and Phenotype of Diarrheagenic *Escherichia coli* Food Product Isolates

No.	*Name*	*Pathotype*	*Virulence genes*	*Serotype O:H*	*MLST seq. type*	*Antibiotic resistance*	*Food product*
1	CCtF019	ETEC	*lt*	128:16	1717	A, AC	Ground beef
2	CCtF047	STEC	*stx-2*	8:19	4495[a]	A, AC	Ground beef
3	CCtF054	STEC	*stx-2*	8:19	1431	A, AC	Ground beef
4	CCtF171.2	ETEC	*lt*	25:12	607	A	Ground beef
5	CCTF197	ETEC	*st*	-:10	10	—	Ground beef
6	CCtF203.1	STEC	*stx-2*	8:28	4496[a]	A, AC	Ground beef
7	CCtF203.2	STEC	*stx-2*	8:28	4496[a]	A, AC	Ground beef
8	CCtF317	EPEC	*eae*	126:+	58	A, AC, T/S	Cheese

[a]New sequence types submitted to the *E. coli* multilocus sequence typing (MLST) database website: http://mlst.warwick.ac.uk/mlst/.
A, ampicillin; AC, amoxicillin/clavulanate; ETEC, enterotoxigenic *E. coli*; STEC, Shiga toxin–producing *E. coli*; T/S, trimethoprim/sulfamethoxazole.

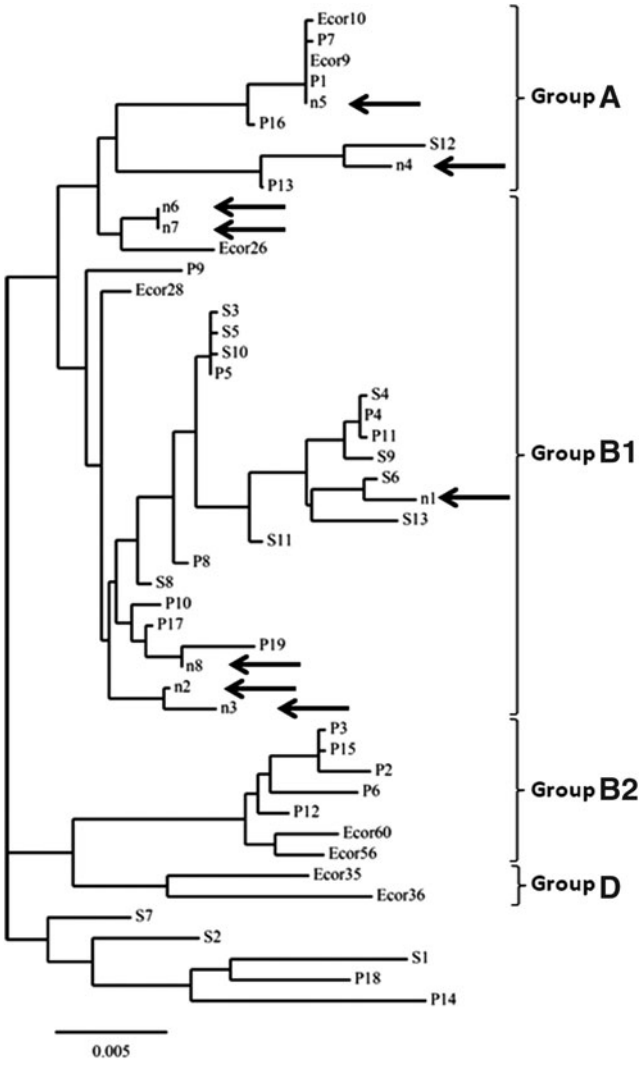

FIG. 1. Multilocus sequence typing (MLST) phylogenetic tree of Shiga toxin–producing *Escherichia coli* (STEC) and enteropathogenic *E. coli* (EPEC) isolates from food products. A phylogenetic tree was constructed by assembly and alignment of MLST DNA sequences using the ClustalW program. *E. coli* strain MLST sequences from ancestral groups A, B1, B2, and D as well as STEC and EPEC sequences, available in the MLST database (http://mlst.warwick.ac.uk/mlst/), were used as controls. STEC controls include the following: S1, STEC 055/89; S2, STEC 493/89; S3, STEC CL/37; S4, STEC DEC11C; S5, STEC 3593/00; S6, STEC 3199/98; S7, STEC 5122/99; S8, STEC 4797/97; S9, STEC E135360; S10, STEC 5244-00-2; S11, STEC 07-07786; S12, STEC 06-EGY30; S13, STEC NIPH-11060424. EPEC controls include: P1, EPEC to HC68; P2, EPEC DEC2A; P3, EPEC 2348/69; P4, EPEC HC10; P5, EPEC HC15; P6, EPEC Trh36; P7, EPEC DEC6A; P8, EPEC DEC12B; P9, EPEC Trh37; P10, EPEC 181; P11, EPEC HC40; P12, EPEC HC36; P13, EPEC HC91; P14, EPEC HC95; P15, EPEC HC59; P16, EPEC HC66; P17, EPEC HC87; P18, EPEC 109; P19, EPEC 219. *E. coli* ancestral control strain sequences were derived from the ECOR collection. Food product STEC and EPEC isolates are represented by n: n2 CCtF047 (STEC); n3, CCtF054 (STEC); n6, CCtF203.1 (STEC); n7, CCtF2203.2 (STEC); n8, CCtF317 (EPEC).

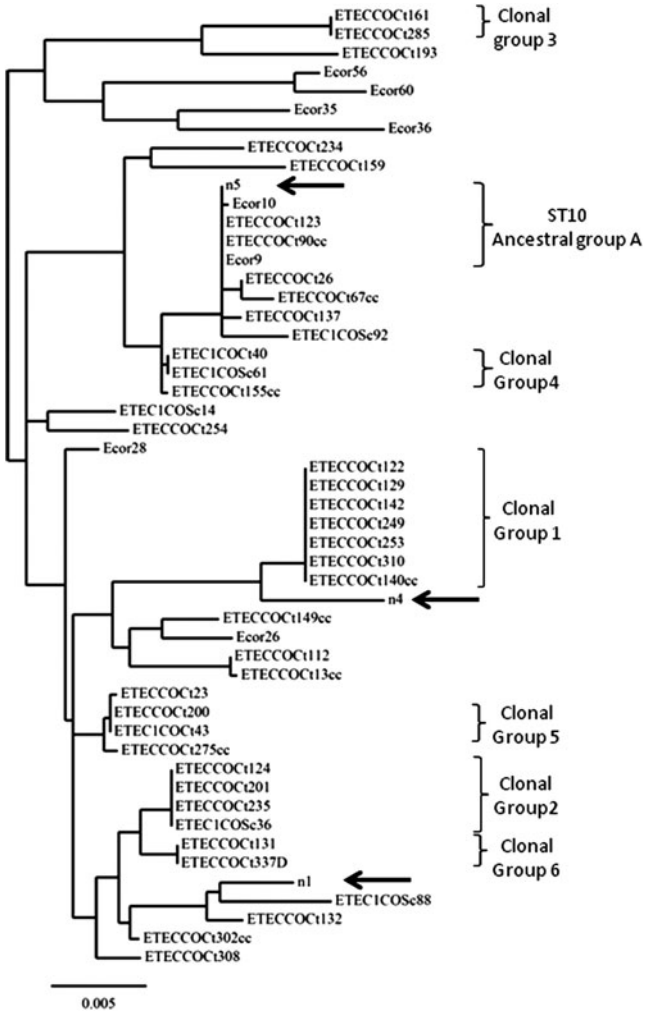

FIG. 2. Multilocus sequence typing (MLST) phylogenetic tree of *Escherichia coli* pathotypes from food products. The phylogenetic tree was constructed by assembly and alignment of MLST DNA sequences using the ClustalW program. *E. coli* strain MLST sequences from ancestral groups A, B1, B2, and D as well as enterotoxigenic *E. coli* (ETEC) sequences, available in the MLST database, were used as controls. *E. coli* ancestral control strain sequences were derived from the ECOR collection. Food product ETEC isolates are represented by n: n1, CCtF019; n4, CCtF171.2; and n5, CCtF197. MLST-based clonal groups 1–6 correspond to human ETEC clinical isolates previously reported (Guerra *et al*., 2014).

contaminated food products was identified among different retail stores. Similarly, no difference was observed in the type of contaminated food products detected per store.

Undercooked ground beef, unpasteurized (raw) milk and juice, soft cheese made from raw milk, and raw fruits and vegetables are frequently contaminated with *E. coli* (Gould *et al*., 2013a, b). These products are important foodborne pathogen vectors in several South American countries (Pires *et al*., 2012). The high frequency of pasteurized milk contamination in retail stores in Cartagena, Colombia may indicate postprocessing contamination or ineffective pasteurization technique. It is of great concern that 48.7% of the pasteurized milk samples were contaminated with coliforms and that 6.6%

were contaminated with *E. coli.* This high bacterial contamination level represents a high risk of foodborne illnesses to the local consumers. It also raises the question of whether milk contamination is widespread in Colombia.

Ground beef was frequently contaminated with coliforms and *E. coli* in this study. Seven (9.2%) of 76 samples were contaminated with pathogenic *E. coli*, including atypical EPEC, STEC, and ETEC. Cheese samples were also often contaminated with coliforms and *E. coli.* However, only one of the samples (1.3%) was contaminated with EPEC. Overall, a total of eight (2%) food product samples were contaminated with pathogenic *E. coli*. Similar *E. coli* pathotypes have been reported in food products from Mexico with the exception of EAEC, which was not identified in our study (Canizalez-Roman *et al*., 2013). Ground beef contamination with STEC is a leading cause of dysenteric diarrhea foodborne outbreaks and HUS (Rivas *et al*., 2006; Werber *et al*., 2012). Countries in Latin America and Europe, in addition to the United States, have reported ground beef contamination with STEC (Brusa *et al*., 2012; Soborg *et al*., 2013; Robbins *et al*., 2014).

Cattle and pork meat contaminated with O157:H7 STEC was reported in Colombia in 1998 (Mattar and Vásquez, 1998). Subsequent studies indicated that STECs detected in food products are predominantly non-O157 STECs serotypes (Martínez *et al*., 2007; Rúgeles *et al*., 2010). O8 was the predominant serogroup among STEC strains isolated from ground beef in this study. While the O8 serogroup has not been associated with cases of human disease in the United States, it has been associated with human illness, including HUS, in Argentina, Germany, and Sweden (Friedrich *et al*., 2002; Johnson *et al*., 2006). O8:H19 is one of the most commonly identified serotypes among STECs recovered from beef in Latin American countries, including Mexico and Argentina (Meichtri *et al*., 2004; Amézquita-López *et al*., 2012).

Detection of ETEC among ground beef samples in Colombia is surprising, as ETEC is frequently recognized as a waterborne pathogen, and less commonly as a foodborne pathogen (Daniels *et al*., 2000). However, the identification of ETEC strains in food products is not very unusual as they have been previously reported in Mexico, Brazil, and Burkina Faso (Echeverria *et al*., 1991; Kagambèga *et al*., 2012; Gómez-Aldapa *et al*., 2014; Ayulo *et al.*, 1994). Here we report that ETEC isolates were detected in three individual ground beef samples. One of the ETEC isolates, ETEC CCtF157 strain, was positive for the ST toxin gene and its MLST sequence was identical to 2 previously reported ETEC clinical isolates from Cartagena (Guerra *et al*., 2014). This isolate was also related to the ECOR9 strain, an ancestral *E. coli* from clonal group A. The remaining ETEC strains were positive for LT. They clustered with previously reported ETEC strains isolated from children with diarrhea in Colombia with MLST analysis. Phylogenetic typing using MLST suggests that ETEC-contaminated ground beef available at retail may contribute to the transmission and elevated ETEC diarrhea morbidity in children living in Cartagena, Colombia.

We identified an atypical EPEC strain positive for *eae* and negative for *bfpA*, from a cheese sample. The EPEC isolate belonged to O126 serogroup that matched to MLST ST58. Although O126 is recognized as one of the classical EPEC O groups (Trabulsi *et al*., 2002), the sequence type ST58 was not only present among EPEC strains but also among EIEC and some extraintestinal pathogenic *E. coli* strains (http://mlst.warwick.ac.uk/mlst/). Atypical EPECs have caused large outbreaks of diarrheal disease in both children and adults (Kaper *et al*., 2004). In industrialized countries, atypical EPEC strains are more frequently isolated from diarrheal cases than typical EPECs expressing the bundle-forming pili.

Antibiotic susceptibility among *E. coli* strains including diarrheagenic and nondiarrheagenic was similar. Most of the isolates were resistant to ampicillin and to amoxicillin/clavulanic acid. Resistance to trimethoprim/sulfamethoxazole was less frequent. This is in contrast to antibiotic multiresistance patterns reported among diarrheagenic *E. coli* strains from food product samples in other Latin American countries including Brazil, Mexico, and Peru (Garcia *et al*., 2011; Canizalez-Roman *et al*., 2013; Pons *et al*., 2014).

In summary, this is the first study of diarrheagenic *E. coli* contamination of the food supply in Cartagena, Colombia. This study highlights the high level of fecal contamination of food products with coliforms and *E. coli* in public markets and the presence of non-O157 STEC and ETEC isolates among food products, particularly ground beef. MLST analysis of diarrheagenic *E. coli* isolates revealed striking identity or similarity with diarrheagenic *E. coli* from children with diarrhea. It suggests that the food products readily available at public markets in Cartagena, Colombia may be involved in the transmission of the diarrheagenic *E. coli* foodborne illnesses. More food product surveillance studies are needed to evaluate the extent of the food product contamination at retail stores in the Colombian cities to measure the frequency and the types of foodborne pathogens. These studies are important for guiding the health policy changes directed at improving food quality and safety during production, processing, and delivery to the public. These policy changes should ultimately help prevent foodborne illness outbreaks, including those associated with high burden of disease and fatal outcomes.

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Disclosure Statement

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