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Isolation and characterization of non-O157 Shiga toxin-producing *Escherichia coli* (STEC) isolated from retail ground beef in Santiago, Chile

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ABSTRACT

Shiga toxin-producing *Escherichia coli* (STEC) is one of the main cause of foodborne disease worldwide, but isolation rates or characteristics of this bacteria from ground beef in Chile are unknown. The present study aimed to isolate and characterize non-O157 STEC from ground beef sold at retail in the city of Santiago, Chile. We analyzed 430 ground beef samples for the presence of STEC, and isolated the microorganism in 10% of samples (43/430). We obtained 56 isolates from the 43 positive samples; 55 of these (98.2%) fermented sorbitol. Most isolates (98.2%; 55/56) showed β-glucoronidase activity, and only six (10.7%; 6/56) were resistant to tellurite. Among the virulence factors studied (stx_1, stx_2, *eae*, and *hlyA*), stx_2 was the only virulence factor in 41% of the isolates (23/56), whereas 10.7% (6/56) of isolates carried a combination of three virulence factors (*stx1* + *stx2* + *hlyA*). None of the isolates carried the gene *eae*. Finally, isolates were neither serogroups O157 nor "big six". In conclusion, ground beef sold in Santiago, Chile is contaminated with STEC; however, further studies are required for understanding their virulence potential.

1. Introduction

Shigatoxin-producing *E. coli* (STEC) is one of the most important foodborne pathogens in the world; the bacterium can cause large outbreaks and severe diseases such as hemolytic uremic syndrome (HUS) and can even cause death (Scallan et al., 2011; Majowicz et al., 2014; WHO, 2015). Bovines are regarded as one of the main reservoirs of the pathogen (Bettelheim, 2007); beef and beef products have been frequently associated with STEC outbreaks (EFSA, 2013; Robertson et al., 2016). STEC O157:H7 has been traditionally linked to human illnesses (EFSA, 2013; Robertson et al., 2016), but over 400 STEC serotypes have been associated with human disease in the world (Blanco et al., 2004; EFSA, 2013). Serogroups O26, O45, O103, O111, O121 and O145—known as the *big six*— are the within most prevalent non-O157 STEC causing disease in the United States (Hoang Minh et al., 2015; USDA, 2012) and in other countries (EFSA, 2013).

STEC strains can carry several virulence factors that are linked to their ability to cause disease. STEC main virulence factors are Shigatoxins Stx1 and Stx2 (encoded by stx_1 and stx_2 genes) and their variants, which interfere with protein synthesis and cause intestinal cell death (Johannes and Römer, 2010). The protein *intimin* (encoded by gene *eae*) is described in highly virulent isolates; it is involved in the close contact between the bacteria and the intestinal cell and the effacing lesions on intestinal mucosal cells (McWilliams and Torres, 2014). HlyA (plasmid gene EHEC-*hlyA*) is an exotoxin that lyses erythrocytes and other cells, promoting iron acquisition for bacterial nutrition (Lorenz et al., 2013). These virulence factors are considered among the main ones involved in STEC pathogenicity.

STEC have been detected and isolated from beef and beef products around the world: In China, STEC was detected in 48% of ground beef and isolated in 9.9% of the samples (Bai et al., 2015; Li et al., 2016); In the United States, non-O157 STEC has been isolated from 5.2% to 7.3% of ground beef samples (Bosilevac and Koohmaraie, 2011; Ju et al., 2012). In Argentina, 25% of beef cuts and 40.7% of minced beef samples were positive for non-O157 STEC at screening (Etcheverría et al., 2010), and non-O157 STEC were

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isolated from 14% of raw ground beef samples (Brusa et al., 2012). In Chile, STEC has been isolated from beef and pork cattle at a slaughterhouse (Borie et al., 1997) and from zoo animals (Marchant et al., 2016). However, the isolation rate of STEC from beef has not been reported. We hypothesized that ground beef is a vehicle for non-O157 STEC in Chile. To test this hypothesis we investigated the presence of STEC in 430 ground beef samples obtained across Santiago, capital of Chile, and determined the presence of the main virulence genes (*stx*$_1$, *stx*$_2$, *eae*, *hlyA*) in the isolates. To characterize the isolates better, we also studied phenotypic characteristics such as sorbitol fermentation, β-glucoronidase activity, resistance to tellurite and production of hemolysin. Finally, we analyzed whether the isolates belonged to some of the most frequent disease-causing serogroups in the world (O157 and big six).

2. Materials and methods

2.1. Sampling

We obtained 430 ground beef samples from grocery stores and butcher shops across the city of Santiago, Chile. Samples were taken biweekly from March to December 2016. The city was divided into 4 main areas (north, west, south and east), and a similar number of samples were taken in each area and from each type of store (Table 1). Samples were transported below 8 °C to the Microbiology and Probiotics Laboratory, University of Chile, and processed the same day.

2.2. Sample processing and screening

Ground beef samples were enriched as previously described (Ju et al., 2012). Briefly, 25 g of ground meat were manually homogenized with 225 ml of modified TSB [30 g TSB (DIFCO) + 1.5 g bile salts N°3 (DIFCO) + 1.5 g K_2HPO_4 (Merck, Germany) for 1 L] in a sterile BagFilter® P bag (Interscience, France). Samples were incubated at 42 °C for 20–22 h. After incubation, 3 loops of the enriched sample were streaked on McConkey agar and incubated at 37 °C for 24 h.

After incubation, DNA was extracted from both the enrichment broth and the McConkey agar plates of each sample, using the InstaGene ™ Matrix (Bio-Rad, Carlsbad, CA) following manufacturer's instructions; two templates for each sample were used to screen for the presence of STEC through a multiple PCR reaction targeting the *stx*$_1$ and *stx*$_2$ genes (Toro et al., 2013). PCR were performed in 25 μl reaction mixture containing 2.5 μl DNA template, 12.5 μl GoTaq® Green Master mix 2X (Promega, Wisconsin), and 0.5 μl (final concentration of 0.2 μM) of each oligonucleotide (IDT, Coralville, IA) (Table 2). The PCR protocol included an initial denaturation step at 95 °C for 5 min, followed by 30 cycles of denaturation (95 °C for 30 s), annealing (56 °C for 30 s) and extension (72 °C for 40 s), with a final extension step at 72 °C for 10 min. PCR products were resolved in 2% (wt/vol) agarose gels on 0.5% TAE buffer at 100 mV for 30 min.

2.3. STEC identification and isolation

When a template tested positive for one or both *stx* genes, 30 individual colonies were examined for the presence of *stx* genes. Positive colonies for the Shigatoxin genes were then confirmed as *E. coli* by a PCR previously described (Chen and Griffiths, 1998) with some modifications. Briefly, the PCR reaction contained 10 μL of GoTaq® Green Master mix 2X (Promega), 0.5 μL (final concentration of 0.3 μM) of each oligonucleotide (IDT; Table 2), 1 μL DNA template and molecular grade water for a final reaction volume of 17 μL. The PCR program included initial denaturation at 94 °C for 5 min, 25 cycles of denaturation (94 °C for 30 s), annealing (58 °C for 30 s) and extension (72 °C for 40 s). The final extension step was at 72 °C for 5 min. STEC isolates (isolates that tested positive for one or both *stx* genes and for *E. coli* by PCR) were stored in 20% glycerol at −80 °C for further analysis.

2.4. Virulence profiling and molecular serogrouping

Each STEC isolate was later characterized for the presence of virulence genes *eae* and *hlyA* by PCR as previously described (Fratamico and Strobaugh, 1998; Xia et al., 2010) (Table 2). DNA was extracted for each individual isolate as described above.

Additionally, we performed a multiplex PCR reaction to determine whether the isolates were of serogroups O26, O45, O103, O111, 0121, 0145, or 157, as described by Toro et al. (Toro et al., 2013) (Table 2). If a band was present, the DNA was tested for each serogroup in individual PCR reactions using the same primers.

DNA from strain ATCC350150 was used as positive control for genes *eae, hlyA* and for serogroup O157. Positive controls for the remaining serogroups were DNA obtained from strains 88–353 (O26), A9619-C2 (O45), B27828/95 (O103), P1338 (O111), SJ18 (O121), and CVM9777 (O145) which were provided by the Laboratory of Food Safety, University of Maryland, College Park, United States.

2.5. Biochemical characterization of STEC isolates

All isolates were characterized for the following biochemical features: a) Sorbitol fermentation test: individual isolates were inoculated on Sorbitol McConkey (SMAC) agar (BD, MD) and incubated at 37 °C. Results were recorded as positive or negative depending on the development of pink colored colonies after 24 h incubation (Miko et al., 2014); b) β-glucoronidase activity test: individual isolates were inoculated on TBX (Tryptone Bile X-glucuronide) chromogenic agar (Biomérieux, France) and incubated at 35 °C and 44 °C for 24 h. Cultures containing the enzyme developed a blue-green color on the agar after incubation (Verhaegen et al., 2015); c) Tellurite resistance was assessed by inoculating isolates on SMAC agar supplemented with 2.5 μg/ml tellurite (CT-SMAC). Plates were incubated at 37 °C for 24 h; cultures with the ability to grow in the media were defined as resistant to tellurite (Miko et al.,

Table 1
Positive samples for STEC at screening and isolation per area in Santiago, Chile.

Type of store	Grocery stores			Butcher shops			Total		
Area	Isolated (%)	Screening (%)	Total samples	Isolated (%)	Screening (%)	Total samples	Isolated (%)	Screening (%)	Total samples
North	7 (13.0)	16 (29.6)	54	8 (14.8)	27 (50)	54	15 (13.9)	43 (39.8)	108
West	3 (5.7)	18 (34.0)	53	5 (9.3)	32 (59)	54	8 (7.5)	50 (46.7)	107
South	4 (7.4)	22 (40.7)	54	5 (9.3)	38 (70.4)	54	9 (8.3)	60 (55.6)	108
East	6 (11.1)	22 (40.7)	54	5 (9.4)	37 (69.8)	53	11 (10.3)	59 (55.1)	107
Total	20 (9.3)	78 (36.3)	215	23 (10.7)	134 (62.3)	215	43 (10.0)	212 (49.3)	430

Statistical comparisons between type of store and area were made using the Pearson Chi-square. No significant differences were detected among isolation rates ($p < 0.05$). All tests were performed in SSPS v25.

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