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STEC non-O157 strains in meat from Southern Sonora, Mexico and their antibiotic resistance

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Abstract

Escherichia coli is one of the most important bacteria associated with the consumption of meat products causing human disease, with bovines as the main reservoir for this organism. *Escherichia coli* serogroups producing the Shiga toxin (referred as STEC) include both O157:H7 and non-O157 strains can cause Hemolytic Uremic Syndrome (HUS). STEC have been previously founded in raw meat sampled at slaughterhouses. The goals of this study were 1) to detect STEC O157 and non-O157 strains in meat cuts and products (beef and pork) in TIF and non-TIF slaughterhouses, as well as butcheries, in southern Sonora, Mexico, and 2) to verify the antibiotic resistance/susceptibility pattern of *E. coli* strains, which contain any toxin-producing gene. To achieve the first aim, two selective media (TS-SMAC agar and Rainbow[®] agar O157) were employed to obtain putative O157:H7 colonies, followed by multiplex PCR for the *stx1*, *stx2*, *eaeA*, and *rfb* genes, using the O157:H7 serotype as a control. No STEC O157 was found in the beef products, but some non-O157 isolates were found. These isolates contained the *stx2* pathogenicity gene (1.40 %, 3/224) and/or the *fliCH4* flagellar antigen (1.78 %, 4/224). This work also confirmed the susceptibility/resistance of strains with the *stx2* and/or *fliCH4* genes to several antibiotics and multi-resistance of all or some of these strains to trimethoprim/sulfamethoxazole, tetracycline, clarithromycin, penicillin G, oxacillin, ampicillin, and cefazolin.

Keywords: STEC non-O157; meat; antibiotic resistance.

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Additional information and declarations
can be found on page 12

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Study contribution

Nowadays, *Escherichia coli* remains as one of main human pathogens contaminating foods. The consumption of beef and pork meat is one of the most important foods for human diet. In this study, two kind of meat samples were evaluated, and some *E. coli* isolates were detected containing *stx2* and/or *fliCH4* genes in beef and pork meat. These isolates were characterized as STEC non-O157:H7 being a potential risk for the consumers. Multiple antibiotic resistance was found in several isolates. This work demonstrates that the beef and pork meat can contains *E. coli* STEC non-O157:H7 representing a risk for the human health. This is the first report in Sonora, Mexico for STEC non-O157:H7.

Introduction

The ability of *E. coli* to cause disease in humans is associated with the production of Shiga toxins (*stx1* and *stx2*).⁽¹⁾ The Shiga toxin-producing *E. coli* (STEC) O157:H7 serogroup was first recognized in 1982 in the US when a massive outbreak of hemorrhagic colitis occurred due to the consumption of contaminated hamburgers,^(2, 3) emerging as an important pathogen for humans.⁽⁴⁾ Beef and swine are asymptomatic carriers of this organism; however, beef is considered the main reservoir of virulent enterohemorrhagic strains.⁽⁵⁾ Once humans ingest this pathogen (infective dose is from 10 to 100 cells/g of food),^(6–8) it takes a 3- to 9-day incubation period before symptoms start to appear, and these symptoms include severe cramping (abdominal pain), diarrhea (initially liquid and later accompanied by bleeding), vomiting, and low or no fever, eventually reaching a stage of permanent renal function loss called Hemolytic Uremic Syndrome (HUS).^(9, 10)

The main routes of transmission of STEC to humans include from person to person through the fecal-oral route,^(1, 6) the consumption of contaminated foods such as unpasteurized milk, water, fruits, vegetables, and raw beef, or through direct contact with animal feces.^(8, 9) In the case of beef, contamination generally occurs during the slaughter of animals and carcass preparation.⁽¹⁰⁾

O157:H7 is the predominant serotype associated with outbreaks and cases of STEC infections worldwide.⁽¹¹⁾ There are a growing number of non-O157 serogroups related to clinical symptoms such as mild to severe diarrhea, hemorrhagic colitis (HC) and even HUS.^(12, 13) This *E. coli* non-O157 serogroup cluster has been termed the “big six”, and includes O26, O45, O103, O111, O121, and O145.⁽¹⁴⁾

The recognized genes to detect O157:H7 are *stx1*, *stx2*, *eaeA*, and *rfb*,⁽¹⁵⁾ while the “big six” serogroup show variations in the genetic composition of the *wzx* gene (O antigen flippase).⁽¹⁶⁾ The *rfb* gene is responsible for the biosynthesis of the O157 antigen, which is specific for detecting the O157:H7 serotype.⁽¹²⁾ The intimin protein, produced by the *eaeA* gene, is important for HUS development and is involved in the process of bacterial adherence to the epithelial cells of the human intestine (attaching and effacing).^(6, 13)

Several studies have demonstrated that the use of antibiotics to treat patients diagnosed with STEC and symptoms of diarrhea or bloody diarrhea may allow the development of HUS. Other reports showed the opposite, the alleviation of HUS symptomatology to milder symptoms.^(14, 17, 18) Besides, *in vitro* studies showed that sub-inhibitory antibiotic concentrations to treating O157:H7 STEC increase the

amount of free Shiga toxin in the culture medium.⁽¹⁸⁾ Although antibiotic prophylaxis or therapy in STEC disease is still not the preferred treatment for patients with STEC, the knowledge on the antibiotic's resistance/susceptibility of the native local STEC strains is always desired.

It is estimated that 112 752 cases of disease associated with this STEC non-O157 cluster occur each year, while O157:H7 is associated with 63 153 cases per year in countries such as Germany, Japan and the US, leading to 20 deaths each year and an annual economic loss of 255 million US dollars.⁽¹⁹⁾ A study performed in the United States over two years that included 4 133 ground beef samples identified 1 006 strains (24.3 %) of STEC carrying *stx1* and/or *stx2*.⁽²⁰⁾ There are few reports for Latin America. 274 half-beef sponges were processed in Argentina; *E. coli* O157 was isolated in four (n:1.4 %), of which two were characterized as *stx2c*(vh-a) *eae/ehx*a, and the other two as non-toxic.⁽²¹⁾ In Santiago, Chile, contamination by non-O157 was reported in 10 % of ground meat samples, with 56 isolates obtained from 43 positive samples showing a 41 % incidence of the *stx2* gene (23/56) and a 10.7 % incidence (6/56) of the combination of the three pathogenicity genes (*stx1*, *stx2*, *hlyA*).⁽²²⁾ In Colombia, the presence of *E. coli* O157:H7 was detected in 0.45 % (n:244) of bovine carcasses and for generic *E. coli* biotype 1 it was 0.89 %.⁽²³⁾ In Mexico, the presence of *E. coli* O157:H7 was detected in 66.66 % (6/9) of beef and pork carcass samples in the Comarca Lagunera,⁽²⁴⁾ while a 5 % incidence (2/40) was detected in beef cuts (rib eye sirloin, New York) in Monterrey, Nuevo León.⁽²⁵⁾ Cabrera-Maldonado et al.⁽²⁶⁾ analyzed 20 samples of ground beef from supermarkets in the city of Puebla and did not detect any O157:H7. In the northwest region of Mexico, where the State of Sonora is located, the incidence of *E. coli* was reported to be 38.24 % (13/34) in ground beef samples taken from Culiacan, Sinaloa, with no detection by real-time PCR of *E. coli* O157:H7 isolates among these samples.⁽²⁷⁾

Mexico produces a high volume of meat (1.88 and 1.32 million tons of beef and pork, respectively) for both consumption and export (8 %-10 %).⁽²⁸⁾ In the State of Sonora, meat production is an important economic activity, and this region has the second and tenth highest pork and beef and pork production, respectively, in Mexico. It is important to conduct studies to detect STEC O157 and non-O157 in this region to prevent disease outbreaks caused by these enteropathogenic bacteria. TIF slaughterhouses in Mexico, are animal slaughter facilities with mechanized processing permanently monitored for their sanitary conditions and regulated by international standards of quality and hygiene. In Mexico, the Secretaría de Agricultura y Desarrollo Rural (Sagarpa) and the standard NOM-194-SSA1-2004 establish the directives for slaughterhouses.⁽²⁹⁾ Although TIF slaughterhouses offer quality meat and optimal sanitary conditions, the non-TIF slaughterhouses do not have mechanized systems and cannot comply with the international quality standards which results on poor quality meat management and suboptimal sanitary conditions.

The goals of this study were 1) to detect STEC O157 and non-O157 *E. coli* in meat products from TIF and non-TIF slaughterhouses, as well as butcherries in southern Sonora, Mexico; and 2) to verify the antibiotic resistance/susceptibility patterns of *E. coli* strains, which contain any toxin-producing gene. We hypothesized that 1) STEC O157:H7 is not present in meat samples in southern Sonora and 2) that the resulting non-O157 isolates will contain multiple resistance patterns to antibiotics.

Materials and methods

Ethical statement

No animal care and use Committee approval was obtained because meat samples were obtained from commercial area of slaughterhouses and in butcheries in the surrounding area to Obregon city in Sonora, Mexico.

Sample collection

Meat samples were collected from two types of slaughterhouses, TIF and non-TIF and from twelve butcheries in the urban area of Ciudad Obregon in Sonora, Mexico. Meat samples from the TIF slaughterhouse were taken from October through November 2015 and from the non-TIF slaughterhouse from October through December 2015 and from 12 local butcheries from March through December 2016, for 190 meat samples. The sampling details (periods, frequency of visits and specific comments on sampling) are provided in Table S1. Fifty-two pork samples were taken from a TIF slaughterhouse (19 loins, 19 ribs, and 14 pulps). A total of 55 beef samples (17 loins, 17 ribs, and 21 pulps) and 33 pork samples (10 loins, 10 ribs, and 13 pulps) were obtained from a non-TIF slaughterhouse. Finally, 50 ground beef samples were obtained from 12 local butcheries. Samples from the TIF slaughterhouses were taken from the freezing chamber (-25 to -30 °C) and the cutting room (4 to 8 °C); and at the non-TIF slaughterhouse samples were collected from the cold room (0 °C) and the cutting room (25 °C). The ground beef samples from the butcheries were collected from refrigerators kept at 4 °C from bulk ground meat containers.

We were unable to conduct a systematic sampling design, instead sampling in this study was transversal, which means that each cut type from the slaughterhouses was sampled at least three times during the sampling period. This type of sampling was performed since the slaughterhouses could not offer visits on a fixed day of the week, or the same type of cut for our weekly visits since this depended on meat availability and the demand for a certain cut type on that day. Nevertheless, we decided to obtain at least five samples of each cut type from the slaughterhouses (Table 1). In the local butcheries, we obtained three to five ground beef samples for ten butcheries, and in a few butcheries a single sample was taken either because they no longer carried out or they ran out of the product at the time of the visit.

Two hundred (slaughterhouses), or fifty (butcheries) grams were collected from each meat sample, placed in a plastic bag, hermetically sealed, and kept refrigerated in coolers at 4 to 8 °C and processed for further analysis in the following 24 hours.

Isolation of presumptive E. coli O157:H7 and non-O157

For isolating presumptive *E. coli* O157 and STEC non-O157 strains twenty-five grams of meat were maintained in 225 mL of Butterfield's phosphate buffer and then shaken vigorously and incubated at 37 °C overnight (enrichment step). Fifty µL of 10⁻² and 10⁻⁴ dilutions were spread in duplicate onto TC-SMAC agar and Rainbow[®] agar O157, and the plates incubated at 37 °C for 18–24 h for screening of typical *E. coli* colonies on TS-SMAC (colorless or neutral/gray with smoky center and 1–2

Table 1. Number of presumptive O157 isolates obtained from meat samples

	Meat cuts	No. of presumptive isolates obtained/ No. samples		
		TIF	non-TIF	Commercial
Pork	CRP	0/7	4/6	-
Pork	CutRP	0/7	17/7	-
Pork	CRR	1/9	4/5	-
Pork	CutRR	0/10	0/5	-
Pork	CRL	2/9	24/5	-
Pork	CutRL	2/10	33/5	-
Beef	CRP	-	5/10	-
Beef	CutRP	-	20/11	-
Beef	CRR	-	10/8	-
Beef	CutRR	-	23/9	-
Beef	CRL	-	23/8	-
Beef	CutRL	-	22/9	-
Beef	*Ground beef	-	-	34/50
Total	Samples	190		
Total	Isolates	224		

CRP= Cold room pulp; CutRP= Cutting room pulp; CRR= Cold room ribs; CutRR= Cutting room ribs; CRL= Cold room loin; CutRL= Cutting room loin.

* Ground beef from local butcheries.

mm in diameter) and on Rainbow[®] agar O157 (black to blue-black colonies).⁽³⁰⁾ Typical colonies were selected and purified by two to three successive passes on chromogenic agar; then, presumptive strains were kept in tryptic soy broth (TSB) (37 °C for 24h) and stored by triplicate in 20 % glycerol at -80 °C until used for biochemical and molecular analysis. The morphological and biochemical characterization were performed in accordance with the Mexican Official Standard.⁽³¹⁾ Isolates, which showed the biochemical profile for *E. coli* (see section below) and the coloration confirmed on Rainbow[®] agar O157 were used for molecular analyses.

Biochemical characterization of isolates

The morphological and biochemical characterization was performed in accordance with Appendix H of the Mexican Official Standard⁽³¹⁾ and included a Gram stain, the oxidase reaction (Merck KGaA, Cat. No. 113300, Darmstadt, Germany), the catalase test, and IMViC. This last test was used to identify enterobacteria; briefly, isolates were incubated in the following: 1) MIO medium (Difco Cat. No. 273520, Le Pont de Claix, France) to demonstrate the production of indole; 2) Methyl Red and Voges-Proskauer broth (Bioxon, Cat. No. 211691, Becton Dickinson de México S.A. de C.V, Cuautitlán Izcalli, México) to analyze the production of carboxylic acids (lactic, succinic, acetic, and formic) from glucose fermentation, as well as to detect the production of acetyl-methyl-carbinol from glucose fermentation to differentiate *E. coli* from the *Klebsiella* and *Enterobacter* genera; and 3) Simmons Citrate agar (Bioxon, Cat. No. 8245170) to determine the usage of citrate as a sole carbon source.⁽³²⁾

Table 2. Primers used to detect pathogenicity and serogroup specific genes

Primer	Sequence (5'- 3')	Amplicon Size (bp)	Pathogenicity / Serogroup gene	Reference
<i>stx1</i> ^F <i>stx1</i> ^R	ATAAATCGCCATTCGTTGACTAC AGAACGCCCACTGAGATCATC	180	Shiga Toxin 1	(33)
<i>stx2</i> ^F <i>stx2</i> ^R	GGCACTGTCTGAAACTGCTCC TCGCCAGTTATCTGACATTCTG	255	Shiga Toxin 2	
<i>eaeA</i> ^F <i>eaeA</i> ^R	GACCCGGCACAAGCATAAGC CCACCTGCAGCAACAAGAGG	384	Intimin (<i>eaeA</i>)	
<i>rfB</i> ^F <i>rfB</i> ^R	TAAGTAATGGAACGGTTGCTCT CCCCACTCGTAAAATCCATC	1000	<i>rfB</i>	(34)
<i>fliCH4</i> ^F <i>fliCH4</i> ^R	ACGGCTGCTGATGGTACAG CGGCATCCAGTGCTTTAAC	244	Flagellar antigen (<i>fliCH4</i>)	(9)

^F=Forward; ^R=Reverse; *wzx*= (O-antigen flippase)

Extraction and quantification of bacterial DNA

Bacterial DNA from STEC O157 presumptive isolates was extracted with the DNA Blood and Tissue kit (Qiagen, Cat. No. 569504, Hilden, Germany) using the protocol for Gram-negative bacteria. The DNA was quantified using a Nanodrop 2000c spectrophotometer (ThermoFisher, Inc., Wilmington, DE, USA). Genomic DNA and PCR products were visualized on 1.5 and 4 % agarose gels, respectively, stained with ethidium bromide (0.33 mg/mL).

The detection of *E. coli* O157:H7 pathogenicity genes by mPCR

Standardization of the mPCR assay for pathogenicity genes was performed using specific primers for the *stx1* (180 bp), *stx2* (255 bp), *eaeA* (384 bp), and *rfB* (1000 bp) genes (Table 2). The reaction mixture was performed in a final volume of 25 µL, which consisted of 1X PCR buffer, 3 mM MgCl₂, 1.25 U of Taq DNA polymerase (Invitrogen, Cat. No. 10342020, Brazil), 0.2 µM of each primer, 0.6 µM dNTPs (Invitrogen, Cat. No. 10297-018, Grand Island, NY, USA), 10–15 ng of DNA, and ultrapure water (Invitrogen, Cat. No. 10977-015) to adjust to the final volume. mPCR reaction conditions were as follows: an initial denaturation step at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 60 °C for 60 s, and extension at 72 °C for 75 s, with a final extension step of 72 °C for 7 min. Genomic DNA from *E. coli* ATCC 25922 was used as a negative control. End-point PCR reactions were performed in a thermocycler (Rosalind 24, Genes2Life SAPI de CV, Irapuato, Guanajuato, México).

Amplicons of the *stx2* gene were purified using a PCR purification kit (Qiagen, Cat. No. 28104, Hilden, Germany) and bidirectionally sequenced using an ABI PRISM 3100 instrument. The *stx2* gene sequences were deposited in the GenBank; JA31 (MH040786), JA49 (MH040787), and JA50 (MH040788).

Identification by PCR of the *fliCH4* gene

The methodology described by Paddock et al⁽⁹⁾ was performed to amplify the flagellar antigen, *fliCH4* (244 bp), with the following modified PCR protocol: an initial denaturation step at 94 °C for 5 min, followed by 35 cycles of denaturation at

94 °C for 30 s, annealing at 57 °C for 45 s, extension at 72 °C for 30 s, and a final extension step at 72 °C for 7 minutes.

Phylogenetic identification using the stx2 gene sequence

Molecular identification was performed using the *stx2* gene sequences obtained from the meat sample isolates. Sequence files were analyzed using SnapGene software (version 4.0.4; SnapGene GLS Biotech LLC, USA). The phylogenetic analysis was performed out using MEGA software version 6.0⁽³⁵⁾ with a partial region of the *stx2* gene (accession number NZ_CP008957.1; 1 355 571-1 355 825 nt) to generate a phylogenetic tree, using reference sequences for *stx2* obtained from GenBank (NCBI). The alignment was performed with the Clustal-W software of MEGA 6.0 and was edited using FigTree v1.4.1. The evolutionary distance was calculated using the Kimura 2 model. The phylogenetic relationship was estimated using the maximum likelihood method. The tree was inferred from 1000 replications (bootstraps).

Susceptibility to antibiotics

Antibiotic tests were performed on isolates positive for the *stx2* and *fliCH4* genes using two techniques. First, the Kirby-Bauer method with Mueller Hinton broth (Difco, Cat. No. 275730, Le Pont de Claix, France) and Mueller Hinton agar (MCD, Cat. No. 7131, Le Pont de Claix, France) was used in accordance with the disk diffusion assay method.⁽³⁶⁾ Diffusion disks impregnated with tetracycline (30 µg), trimethoprim/sulfamethoxazole (25 µg, 1:19 w/w)⁽¹⁸⁾, penicillin G (10 IU), oxacillin (1 µg), or clarithromycin (15 µg) (Oxoid, Cat. Nos. CT0043B, CT0159B, CT0054B, CT0693B, and CT0052B, respectively) were used. *E. coli* O157:H7 was used as a test control. The Kirby scale was used to evaluate susceptibility/resistance to antibiotics by measuring the growth inhibition halos of the bacterial isolates.⁽³⁶⁾ The criteria used to read the inhibition halos were as follows: if the halo was $\geq$ 16-mm isolates were susceptible, if the halo was between 11–15-mm susceptibility was considered intermediate, and if the halo was $\leq$ 10 mm⁽³⁷⁾ isolates were considered resistant to that specific antibiotic. The assay was performed in triplicate and the diameter of the growth inhibition area around the disks (mm) was determined after 18 h of incubation at 37 °C.

Additionally, a second methodology to confirm susceptibility/resistance to these antibiotics was used. An analysis of minimum inhibitory concentration (MIC, µg/mL) was carried out with 68 Microscan GN systems according to the Clinical and Laboratory Standard Institute (CLSI).

Results and discussion

Isolation of O157 presumptive isolates

This study focused on establishing the presence of STEC O157:H7. Table 1 shows that presumptive isolates from pork samples from the TIF slaughterhouse were low compared to isolates found in the non-TIF slaughterhouse, this is possibly due to higher temperatures used in the cold and cutting rooms of the non-TIF (0 and 25 °C) than in those of the TIF (-25 to -30; 4 to 8 °C) slaughterhouse.

Table 3. *Escherichia coli* genes detected in isolates from beef and pork

	Genes					
Controls	<i>rfB</i>	<i>stx1</i>	<i>stx2</i>	<i>eaeA</i>	<i>fliCH4</i>	<i>wzx</i> O157
O157:H7	+	+	+	+	-	+
ATCC 25922	-	-	-	-	-	-
Isolates						
JA31	-	-	+	-	+	-
JA49	-	-	+	-	-	-
JA50	-	-	+	-	-	-
JA107	-	-	-	-	+	-
JA113	-	-	-	-	+	-
JA116 ^{&}	-	-	-	-	+	-

All isolates were gram-negative short bacilli; [&] isolated from pork.

There was a tendency to find a higher number of isolates in the pork and beef samples from the cut rooms, mainly in the non-TIF samples. The number of isolates was highest from pork loins in both the TIF and non-TIF slaughterhouses. Beef samples in the non-TIF slaughterhouse were also highest in the loin cuts compared to the pulp or rib cuts.

However, even when 224 presumptive isolates were obtained as presumptive STEC O157:H7 we were unable to confirm their identity by PCR as belonging to this serotype (described in the following section). After this finding, the study was re-focused on the characterization of these *E. coli* non-O157 isolates (Table 1). There are few reports on STEC in Mexico; however, studies of ground beef samples from Culiacan, Sinaloa⁽²⁷⁾ and from supermarkets in the city of Puebla, Puebla⁽²⁶⁾ did not find *E. coli* O157:H7, which was consistent with our findings.

Molecular characterization of O157 presumptive isolates

Standardization of the mPCR assay for pathogenicity genes is shown in Figure S1. Multiplex PCR showed that the presence of the *stx2* pathogenicity gene was 1.40% (3/224). Isolates that were positive for this gene were JA31, obtained from a beef loin sample from a non-TIF slaughterhouse and isolates JA49 and JA50, obtained from beef pulp samples from the same non-TIF slaughterhouse (Table 3, Figure S2). All three isolates were from beef and together showed 2.9% (3/105) for the *stx2* gene. Positive amplicons for *stx2* were sequenced to confirm their identity. The sequences obtained for *stx2* from isolates JA31, JA49, and JA50 were identical, so a single sequence was selected to build the phylogenetic tree (Figure 1), which shows that some O157:H7 strain cluster together with strains from serogroups O104:H4 and O145:H28 on a different branch of the tree, but that these strains are closer to the branch where JA31 is located than to the branch comprising other nonpathogenic *Escherichia* species. Phylogenetic analysis confirmed that the isolates from which the *stx2* gene was positively amplified belonged to the *stx2* gene from other *E. coli* serogroups reported.

In the phylogenetic tree, the isolate JA31 was clustered with pathogenic strains that have caused clinical outbreaks (Figure 1). The KJ158456.1 sequence

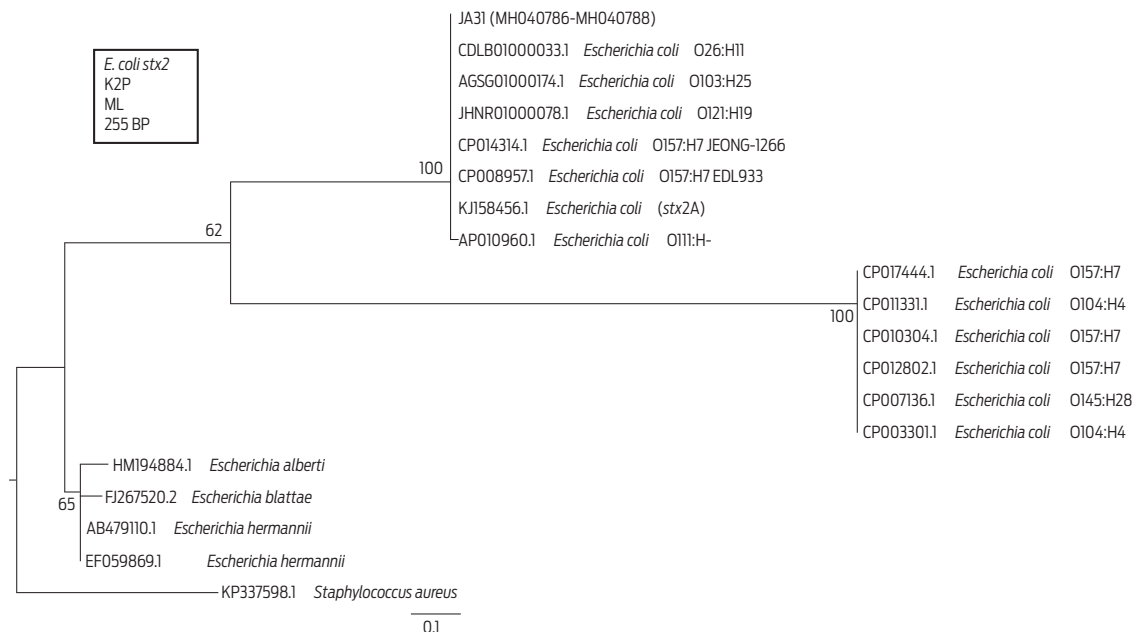


Figure 1. Distance tree derived from partial sequences of the *stx2* gene. The tree was built with Mega 6 software, using the Kimura 2 parameter (K2P) substitution model. Branches show the percentages corresponding to 1 000 bootstrap replicates. GenBank accession numbers are found at the start of each name. The representative sequences of the three isolates from this assay are designated as JA31 (MH040786-MH040788). The scale bar indicates a genetic distance of 0.1 for each nucleotide substitution per site of the *stx2* sequence. The KP337598.1 sequence from *S. aureus* was used as an outgroup to root the tree. ML= Maximum likelihood.

belongs to a strain from a patient with bloody diarrhea and was identified as non-motile *E. coli* O59:H⁺, which possesses the *stx2* gene, as well as other genes for type IV aggregated adherent fimbriae.⁽³⁷⁾ Isolate JA31 was also clustered with the AGSG01000174.1 sequence from an entero-hemorrhagic *E. coli* (EHEC) strain of STEC O103:H25 that produced a severe outbreak that caused HUS due to contaminated food in Norway in 2006.⁽¹⁵⁾

Stx2 was the most frequently found gene in studies of *E. coli* non-O157 in ground beef samples collected from Chile.⁽²²⁾ The *stx2* toxin is 400 times more lethal than *stx1* and plays a critical role in HUS development.⁽³⁸⁾ There are reports of outbreaks associated with other serogroups that cause HUS^(39, 40) in countries such as Argentina and Germany^(6, 37, 41) by pathogens such as *E. coli* O104:H4, which has the *stx2* and *fliCH4* genes, but does not possess the *stx1*, *eaeA*, or *rflB* genes.⁽⁴²⁾ Based on this information, we decided to analyze our isolates for the presence of the *fliCH4* gene characteristic of the O104:H4 serogroup⁽⁹⁾ (Table 3).

Figure S3 and Table 3 show that isolates JA31 (beef loin), JA107 (beef ribs), JA113 (beef ribs) and JA116 (pork pulp), all from the non-TIF slaughterhouse, were positive for the *fliCH4*. These data indicated 2.9% (3/103) and 1.2% (1/82) presence of the *fliCH4* gene in beef and pork samples, respectively. The isolate JA31 in this assay, from the non-TIF slaughterhouse beef loin, had two genes, *stx2* and *fliCH4*, to serogroup O104:H4, which shows characteristics of both enteroaggregative *E. coli* (EAEC) and enterohemorrhagic (EHEC).⁽³⁷⁾ However, the *wzx* gene-specific for O104 was not amplified by PCR in this isolate, so we disregarded this possibility.

In this region of Mexico, there are no reports of *E. coli* containing the *stx2* or *fliCH4* genes from beef samples. Our group has recently reported the presence of *E. coli* O157:H7 in fecal samples of asymptomatic cattle in Sonora.⁽⁴³⁾ Hence, con-

ducting an in-depth study of JA31 to help understand the reason for the presence of these genes on a local scale is relevant. This knowledge may help understand *E. coli* disease outbreaks both locally and globally, possibly helping prevent global problems caused by STEC in the future.

In Mexico, the presence of *E. coli* O157:H7 has been reported in beef and pork carcasses in Northern Mexico, with an incidence of 66.6% (12/18).⁽²⁴⁾ In another work, the incidence of *E. coli* O157:H7 (2.7%) and non-O157 (5%) strains were reported from 258 samples taken from beef carcasses in a non-TIF slaughterhouse in Guadalajara. In this work, the incidence of *E. coli* non-O157 was only 1.40% (out of 224 samples) from beef and pork carcasses. This rate was lower than those reported at the non-TIF slaughterhouse in Guadalajara.⁽⁴⁴⁾

The JA31 isolate possessed the *stx2* and *fliCH4* genes. Within seropathotype D, there are O113:H4 and O7:H4 strains, and JA31 may be a candidate to demonstrate the presence of the *wzx* gene in serogroup O7 because we ruled out the possibility that JA31 is an O113 since this strain did not amplify with *wzx* genes for O113 (data not shown). The presence of these genes (*stx2* and *fliCH4*) in the isolates in this study does not guarantee the expression and production of the Shiga toxin. Thus, further confirmation by evaluating the presence of the toxin in these isolates is needed.

Susceptibility and/or resistance to antibiotics

The isolates identified as *E. coli* that tested positive for the *stx2* gene (3/224) and/or the *fliCH4* gene (4/224), displayed resistance to tetracycline, oxacillin, penicillin G, ampicillin, sulbactam, cefazolin and trimethoprim/sulfamethoxazole while being susceptible to the other 16 antibiotics tested (Table 4). The main resistance mechanism displayed by these isolates belongs to beta-lactamase inhibitors, and tetracyclines affecting the bacterial protein synthesis.⁽⁴⁵⁾ Such multiple-resistance to antibiotics has been previously reported in STECs.⁽⁴⁶⁾

The combination of antibiotics is recommended to treat a range of human infections, including urinary tract infections, uncomplicated acute cystitis, and pyelonephritis. Nevertheless, the use of antibiotics in individuals with STEC infections is controversial and generally not recommended, this is because some antibiotics affect DNA replication by activating the SOS response in bacteria and indirectly increasing the number of toxins such as ST.^(17, 18) This study confirms the variability concerning the susceptibility or resistance to antibiotics of *E. coli* strains containing the *stx2* and/or *fliCH4* genes.

Conclusions

This study reports that no *E. coli* STEC O157:H7 was found in 224 fresh beef and pork meat samples taken from one TIF and one non-TIF slaughterhouse and 12 local butcherries in Ciudad Obregon, Sonora. Nevertheless, the *stx2* and *fliCH4* genes were present in some isolates from beef meat at low frequencies (1.40 and 1.78%). Isolates containing the *stx2* and/or *fliCH4* gene showed multiple antibiotic resistance to tetracycline, penicillin G, oxacillin, ampicillin, sulbactam, cefazolin and trimethoprim/sulfamethoxazole.

Table 4. Antibiotic susceptibility of *Escherichia coli* isolates with *stx2* or *fliCH4* genes from meat products

Method	Antibiotic	Dose per disk	JA31	JA49	JA50	JA107	JA113	JA116
Minimum inhibitory concentration **MIC (µg/mL)	AK		s < 16	s < 16	s < 16	s < 16	s < 16	s < 16
	Aug		s < 8/4	s < 8/4	s < 8/4	s < 8/4	s < 8/4	s < 8/4
	A/S		r > 16/8	r > 16/8	r > 16/8	r > 16/8	r > 16/8	r > 16/8
	AM		r > 16	r > 16	r > 16	r > 16	r > 16	r > 16
	Cfz		r > 4	r > 4	r > 4	r > 4	r > 4	r > 4
	Cpe		s < 4	s < 4	s < 4	s < 4	s < 4	s < 4
	Cft/CA		s < 0.5	s < 0.5	s < 0.5	s < 0.5	s < 0.5	s < 0.5
	Cft		s < 2	s < 2	s < 2	s < 2	s < 2	s < 2
	Caz		s < 1	s < 1	s < 1	s < 1	s < 1	s < 1
	Caz/CA		s < 0.25	s < 0.25	s < 0.25	s < 0.25	s < 0.25	s < 0.25
	Cax		s < 1	s < 1	s < 1	s < 1	s < 1	s < 1
	Crn		s < 4	s < 4	s < 4	s < 4	s < 4	s < 4
	Cp		s < 1	s < 1	s < 1	s < 1	s < 1	s < 1
	Etp		s < 0.5	s < 0.5	s < 0.5	s < 0.5	s < 0.5	s < 0.5
	Gm		s < 2	s < 2	s < 2	s < 2	s < 2	s < 2
	Imp		s < 1	s < 1	s < 1	s < 1	s < 1	s < 1
	Lvx		s < 2	s < 2	s < 2	s < 2	s < 2	s < 2
	Mer		s < 1	s < 1	s < 1	s < 1	s < 1	s < 1
	P/T		s < 16	s < 16	s < 16	s < 16	s < 16	s < 16
	Te		r > 8	r > 8	r > 8	r > 8	r > 8	r > 8
	Tgc		s < 2	s < 2	s < 2	s < 2	s < 2	s < 2
	To		s < 4	s < 4	s < 4	s < 4	s < 4	s < 4
	T/S		r > 2/38	r > 2/38	r > 2/38	r > 2/38	r > 2/38	r > 2/38
Kirby–Bauer (Inhibition halos in mm)	Te	30 µg	0.0 (r)	0.0 (r)	0.0 (r)	0.0 (r)	0.0 (r)	0.0 (r)
	FOS	200 µg	≥16 (s)	≥16 (s)	≥16 (s)	≥16 (s)	≥16 (s)	≥16 (s)
	CLR*+	15 µg	10.0 (r)	12.9 (is)	14.0 (is)	0.0 (r)	0.0 (r)	0.0 (r)
	OX*+	1 µg	0.0 (r)	0.0 (r)	0.0 (r)	0.0 (r)	0.0 (r)	0.0 (r)
	P*+	10 iu	0.0 (r)	0.0 (r)	0.0 (r)	0.0 (r)	0.0 (r)	0.0 (r)

** 68 Microscan GN systems; MIC = minimum inhibitory concentration according to Clinical and Laboratory Standard Institute criteria.

AK, amikacin; Aug, amoxicillin-clavulanate; A/S, ampicillin-sulbactam; AM, ampicillin; Cfz, cefazolin; Cpe, cefepime; Cft/CA, cefotaxime-clavulanate; Cft, cefotaxime; Caz, ceftazidime; Caz/CA, ceftazidime-clavulanate; Cax, ceftriaxone; Crn, ceforoxime; Cp, ciprofloxacin; ETP, ertapenem; Gm, gentamicin; Imp, imipenem; Lvx, levofloxacin; Mer, meropenem; P/T, piperacillin-tazobactam; Te, tetracycline; TGC, tigecycline; To, tobramycin; T/S, trimethoprim-sulfamethoxazole; Te, tetracycline; T/S, trimethoprim/sulfamethoxazole; FOS, fosfomicin; CLR, clarithromycin; OX, oxacillin; P, penicillin G; IU= international unit.

* Isolates are susceptible (S) to an antibiotic when they show an inhibition halo ≥ 16 mm, intermediate susceptible (IS) when they show an inhibition halo between 11 and 15 mm, and resistant (R) to an antibiotic if the halo is ≤ 10 mm. +antibiotic not included in the Clinical and Laboratory Standards Institute for *Enterobacteriaceae*.

Data availability

The partial region of the *stx2* gene were deposited in the NCBI database with accession number NZ_CP008957.1; 1 355 571-1 355 825 nt.

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Conflicts of interest

The authors have no conflict of interest to declare in regard to this publication.

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