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Research Note

Genetic Characterization of *Escherichia coli* Isolated from Cattle Carcasses and Feces in Mexico State

NYDIA E. REYES-RODRÍGUEZ,[1] EDGARDO SORIANO-VARGAS,[1] JEANNETTE BARBA-LEÓN,[2] ARMANDO NAVARRO,[3] MARTÍN TALAVERA-ROJAS,[1] A. MARIEL SANSO,[4] AND ANA V. BUSTAMANTE[4]*

[1]*Centro de Investigación y Estudios Avanzados en Salud Animal, Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de México, Toluca, Estado de México, México;* [2]*Departamento de Salud Pública, Centro Universitario de Ciencias Biológicas y Agropecuarias, Universidad de Guadalajara, Zapopan, Jalisco, México;* [3]*Departamento de Salud Pública, Universidad Nacional Autónoma de México, Ciudad Universitaria, México; and* [4]*Consejo Nacional de Investigaciones Científicas y Técnicas, Laboratorio de Inmunoquímica y Biotecnología, Centro de Investigación Veterinaria de Tandil, Facultad de Ciencias Veterinarias, Universidad Nacional del Centro de la Provincia de Buenos Aires, 7000 Tandil, Argentina*

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ABSTRACT

Meat of bovine origin is one of the major vehicles in the transmission of verotoxigenic *Escherichia coli* (VTEC) to human consumers. This pathogen can produce serious human illness, including bloody diarrhea and hemolytic uremic syndrome. The aim of the current study was to characterize *E. coli* isolates (mainly VTEC strains) belonging to several serotypes in samples from cattle carcasses and feces of three municipal slaughter plants from Mexico State. The genetic diversity and molecular relatedness among the isolates was evaluated with multiple-locus variable-number tandem repeat analysis (MLVA). To our knowledge, and with the exception of *E. coli* O157:H7, this is the first time that serotypes analyzed here have been subtyped by MLVA in Mexico. MLVA typing grouped the 37 strains from this study into 30 distinct genotypes, 26 of which were unique. These findings indicate that cattle carcasses and feces from slaughter plants in Mexico are a source of VTEC that are genetically diverse in terms of serotypes and virulence profiles. The presence of these pathogens in carcasses indicates the high probability of the spread of VTEC strains during slaughter and processing.

Escherichia coli can colonize the gastrointestinal tract of humans and animals without causing damage *(40)*; however, verotoxigenic *E. coli* (VTEC), also known as Shiga toxigenic *E. coli,* is an important foodborne pathogen that can cause severe diseases in humans. The infection symptoms can include bloody diarrhea, abdominal pain, and in some cases neurologic and renal complications, such as hemolytic uremic syndrome *(17, 37)*.

VTEC is characterized by the production of verotoxins (VT1 and VT2) encoded by the vtx_1 and vtx_2 genes, respectively *(15)*. Other virulence factors that can be found in these strains are intimin (an outer membrane protein) encoded by the *eae* gene *(14)* and implicated in attachment to the intestinal epithelial cells, an enterohemolysin encoded by the *ehxA* gene *(16),* which liberates hemoglobin from the red blood cells during infection and has been linked to severe disease symptoms, and the Shiga toxigenic *E. coli* (STEC) autoagglutinating adhesin encoded by the *saa* gene on a plasmid *(33)*.

VTEC has been implicated in outbreaks associated mainly with zoonotic origin, and cattle and their products are considered to be the major vehicle of this pathogen *(8, 25)*. Meat becomes contaminated at the time of slaughter, and processing performed under nonhygienic conditions may introduce the bacteria *(10)*. Contamination of carcasses with VTEC can occur when gut contents or fecal matter contact the meat surfaces, and cross-contamination between carcasses may occur during processing *(5)*.

Studies of VTEC prevalence in bovine feces and carcasses of selected beef exporting abattoirs of Argentina revealed that VTEC non-O157 was present in 22.3 and 9.0% of the feces and carcasses, respectively *(23),* and the prevalence of VTEC O157 was 4.1 and 2.6%, respectively *(24)*. In another study, 12.34 and 18.64% of VTEC in carcasses were detected at the slaughter area and sanitary control cabin, respectively *(6)*.

In Mexico, few studies have been conducted to determine the presence of VTEC strains and their virulence factors in beef carcasses and domestic animal feces. Amézquita-López et al. *(1)* detected 12.5% VTEC prevalence (5.4% O157 and 7.1% non-O157) in feces of animals from small rural farms in the Culiacan Valley in northwestern Mexico, and Callaway et al. *(4)* and Narvaez-Bravo et al. *(26)* found 3.35 and 23.2% prevalence of VTEC O157:H7, respectively, in farm animals. The contamination of beef carcasses with VTEC O157:H7 and non-O157 was detected in slaughter plants in the city of Guadalajara, with prevalences of 20.5% for *E. coli* non-O157, 5% for *E. coli* O157:NM, and 2.7% for *E. coli*

* Author for correspondence. Tel: +54-249-4439850, Int 275; Fax: +54-249-4439850; E-mail: avbustaman@vet.unicen.edu.ar.

TABLE 1. *Virulence genotypes of VTEC serotypes isolated from cattle carcasses and feces in Mexico State slaughter plants*

Serotype	Isolate(s)	Sample type (source[a])	Virulence genotype:				
			vtx$_1$	*vtx*$_2$	*eae*	*ehxA*	*saa*
O7:H30	55	Feces (C)	−	+	+	+	−
O8:H25	43	Carcass (C)	+	+	+	+	−
O18:H21 ($n = 2$)	39	Carcass (C)	−	+	−	−	−
	44	Feces (C)	−	+	−	−	−
O22:H8 ($n = 6$)	45, 47	Carcass (C)	−	+	−	−	−
	46	Feces (C)	−	+	−	−	−
	48	Feces (C)	+	+	−	−	−
	50	Carcass (C)	+	+	−	−	−
	51	Feces (C)	+	−	−	−	−
O37:H10 ($n = 2$)	3	Feces (A)	+	+	−	+	−
	6	Feces (A)	−	−	−	−	−
O40:NM	7	Carcass (A)	−	+	+	+	−
O112:H2 ($n = 3$)	2	Carcass (A)	+	+	−	−	−
	19	Carcass (B)	−	+	−	−	−
	34	Feces (C)	−	−	−	−	−
O117:H4 ($n = 2$)	56	Feces (C)	−	+	−	−	−
	36	Feces (C)	−	−	−	−	−
O118:H21	21	Carcass (C)	+	+	+	+	−
O136:H16	52	Carcass (C)	−	+	−	−	−
O154:H53 ($n = 2$)	32	Carcass (C)	+	−	−	−	−
	33	Carcass (C)	−	−	−	−	−
O157:H7 ($n = 6$)	3A, 4A, 5A, 6A, 23	Carcass (C)	+	+	+	+	−
	38	Feces (C)	+	+	+	+	−
O165:H16	1	Carcass (A)	+	+	−	−	−
O185:H7	13	Feces (B)	−	+	−	−	−
ONT:H51 ($n = 2$)[b]	25	Carcass (C)	−	+	−	+	−
	28	Feces (C)	−	−	−	−	−
OR:H7 ($n = 3$)[c]	1A, 2A, C	Carcass (C)	+	+	+	+	−
OR:HNT ($n = 2$)	17	Feces (B)	−	−	−	+	−
	G	Carcass (C)	−	+	−	−	−

[a] Slaughter plant A, B, or C.
[b] NT, nontypeable.
[c] R, rough.

O157:H7 *(39)*. The prevalence of *E. coli* O157:H7 in the Central Mexican Plateau was 2.6% *(34)*.

Multiple-locus variable-number tandem repeat analysis (MLVA) is a subtyping method that allows the characterization of VTEC and other bacteria *(19)* and the discrimination between isolates based on the number of tandem repeat sequences of different loci with a variable number of tandem repeats (VNTR). The set of alleles at all loci analyzed results in a characteristic MLVA profile for a given genetic isolate. Thus, it is possible to differentiate isolates that would be indistinguishable by other methods *(18–21, 27–29)*.

The aim of the current study was to characterize VTEC strains isolated from cattle carcasses and feces in three municipal slaughter plants in Mexico State and to ascertain the genetic diversity and molecular relatedness among these isolates using MLVA.

MATERIALS AND METHODS

Bacterial isolates. A total of 37 *E. coli* isolates (31 VTEC and 6 non-VTEC) from the collection of the Centro de Investigación y Estudios Avanzados en Salud Animal (Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de México) were investigated. The non-VTEC isolates were included in this study to compare them with VTEC isolates of the same serotype. The isolates had been obtained from cattle carcasses and feces in three municipal slaughter plants (A, B, and C) located in México State during 2009. These isolates belonged to 17 serotypes (Table 1), as previously determined with serotyping agglutination assays *(30)* using 96-well microtiter plates and rabbit sera (SERUNAM, Mexico City, Mexico) obtained against 187 somatic antigens and 53 flagellar antigens for *E. coli* and against 45 somatic antigens for *Shigella* species.

DNA extraction. Suspensions of bacterial cells were boiled for 10 min and used directly in the PCRs *(2)*.

Virulence profile. All isolates were analyzed by PCR for the presence of *vtx*$_1$, *vtx*$_2$, *eae, ehxA,* and *saa* genes using the primers and conditions described by Paton and Paton *(31, 32)*. PCR products were visualized by electrophoresis on 2% agarose gels stained with ethidium bromide.

MLVA typing. Seven VNTR loci were amplified using the primers described by Lindstedt et al. *(19)*. The assay was

TABLE 2. *Characterization of the seven VNTR (variable-number tandem repeat) loci used in this study*

Parameter	VNTR loci:						
	CVN001	CVN002	CVN003	CVN004	CVN007	CVN014	CVN015
Repeat unit size (bp)	39	18	15	15	6	6	6
No. of alleles	5	9	4	5	6	10	3
Allelic range (no. of tandem repeats)	0–7	1–10	4–7	6–11	3–9	3–13	5–8
Null alleles	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Nei's diversity index (D_N)	0.54	0.82	0.45	0.75	0.65	0.80	0.38

performed as described by Bustamante et al. *(3)* in two multiplex PCR runs, MI (loci CVN007, CVN014, and CVN015) and MII (loci CVN001 and CVN004), and two monoplex PCR runs (loci CVN002 and CVN003). PCR products were analyzed by electrophoresis on 6% denaturing polyacrylamide gels, and bands were detected by silver staining (Silver Stain Detection, GenePrint STR Systems, Promega, Madison, WI). The different allelic variants detected for each VNTR were sequenced with an ABI PRISM 3730 XL analyzer (Macrogen, Seoul, Korea). The sequences were analyzed using Chromas 2.32 software (Technelysium Pty Ltd., Tewantin, Australia).

Data analysis. The alleles observed were named according to the number of tandem repeats. Absence of amplification product was considered a null allele *(19, 28).* Nei's diversity index (D_N), based on Nei's marker diversity, was calculated for each locus according to Bustamante et al. *(3).* MLVA profiles were defined in the following locus order: CVN001, CVN002, CVN003, CVN004, CVN007, CVN014, and CVN015 *(19).* The dendrogram was constructed using the unweighted pair group method with arithmetic means clustering method implemented by START v. 1.0.5 software *(13).* The discriminatory power of the typing method was evaluated using Simpson's index of diversity (D_S) *(12).*

RESULTS

A total of 37 *E. coli* strains were characterized in this study (31 VTEC and 6 non-VTEC). Among the VTEC strains analyzed, 11 (35.5%) carried vtx_1, vtx_2, *eae,* and *ehxA*, 10 (32.2%) carried only vtx_2, 4 (12.9%) carried vtx_1 and vtx_2, 2 (6.5%) carried vtx_2, *eae,* and *ehxA*, 2 (6.5%) carried only vtx_1, 1 (3.2%) carried vtx_1, vtx_2, and *ehxA,* and 1 (3.2%) carried vtx_2 and *ehxA*. The STEC autoagglutinating adhesion gene (*saa*) was not detected (Table 1).

All the isolates tested could be typed with this MLVA, and a total of 42 alleles were found. The number of alleles detected per locus ranged from 3 (locus CVN015) to 10 (locus CVN014). The number of repeats identified in this collection ranged from 0 to 13 among the seven loci analyzed (Table 2). All the loci had null alleles (Table 2), suggesting either locus absence or sequence polymorphism at one or both of the priming sites for some isolates.

Diversity indexes were calculated for all VNTRs using Nei's index. CVN015 had the lowest value ($D_N = 0.38$), whereas CVN002 and CVN014 were the most polymorphic loci ($D_N = 0.82$ and 0.80, respectively) (Table 2). The isolates could be principally discriminated by alleles of loci CVN014 and CVN002, followed by those of CVN004. Simpson's index of diversity was calculated for the combined typing set: $D_S = 0.98$.

The MLVA dendrogram had two main clusters, one corresponded to non-O157 isolates and the other corresponding to O157:H7 and OR:H7 isolates (Fig. 1). The total strains were divided into 30 distinct genotypes, 26 of which were unique to a single strain (Fig. 1). No profile was shared by isolates belonging to different serotypes. The most frequent MLVA profile was 7-9-30-30-4-8-5, found for five isolates (13.5%) belonging to serotype O22:H8. All of these isolates came from the same slaughter plant (C); however, two different virulence profiles were detected among them: vtx_2 ($n = 3$) and vtx_1/vtx_2 ($n = 2$). The other three MLVA profiles were shared between two isolates belonged to O37:H10, O157:H7, and OR:H7, respectively (Fig. 1). For the O37:H10 serotype, both isolates had the same origin (plant A) but different virulence profiles, whereas for serotypes O157:H7 and OR:H7 the isolates were from the same sampling site (plant C) and had the same virulence factors.

Because *vtx*-positive and *vtx*-negative isolates were detected in serotype O37:H10, O112:H2, O117:H4, O154:H53, and OR:HNT isolates, all of these isolates were included in the analysis. For the O37:H10 serotype, both VTEC and non-VTEC isolates had the same MLVA profile, but this was not the case for the other serotypes.

DISCUSSION

We compared *E. coli* isolates (mainly VTEC isolates) belonging to several serotypes that were collected from cattle carcasses and feces in Mexican slaughter houses. Some of these serotypes, such as O157:H7, O22:H8, and O117:H4, have been previously associated with human infections *(11, 36, 41).* Serotype O22:H8, one of most prevalent serotypes in this study, is also one of the VTEC serotypes most frequently detected from bovine meat and products and has been described as one of the most frequent colonizers of cattle worldwide *(22).*

MLVA was successfully applied to analyze the genetic diversity among the studied isolates. The discriminatory power evaluated by Simpson's index of diversity resulted in a value that can be considered highly discriminatory ($D_S = 0.98$). To our knowledge, only one previous study has characterized VTEC strains from Mexico by MLVA *(1),* and the current study is the first in which non-O157:H7 VTEC isolates from Mexico have been subtyped by MLVA.

Amplification of the CVN003 locus has been reported in isolates from a few serotypes, such as O145:NM and O157:H7 *(3, 19),* in isolates from serotypes positive for the

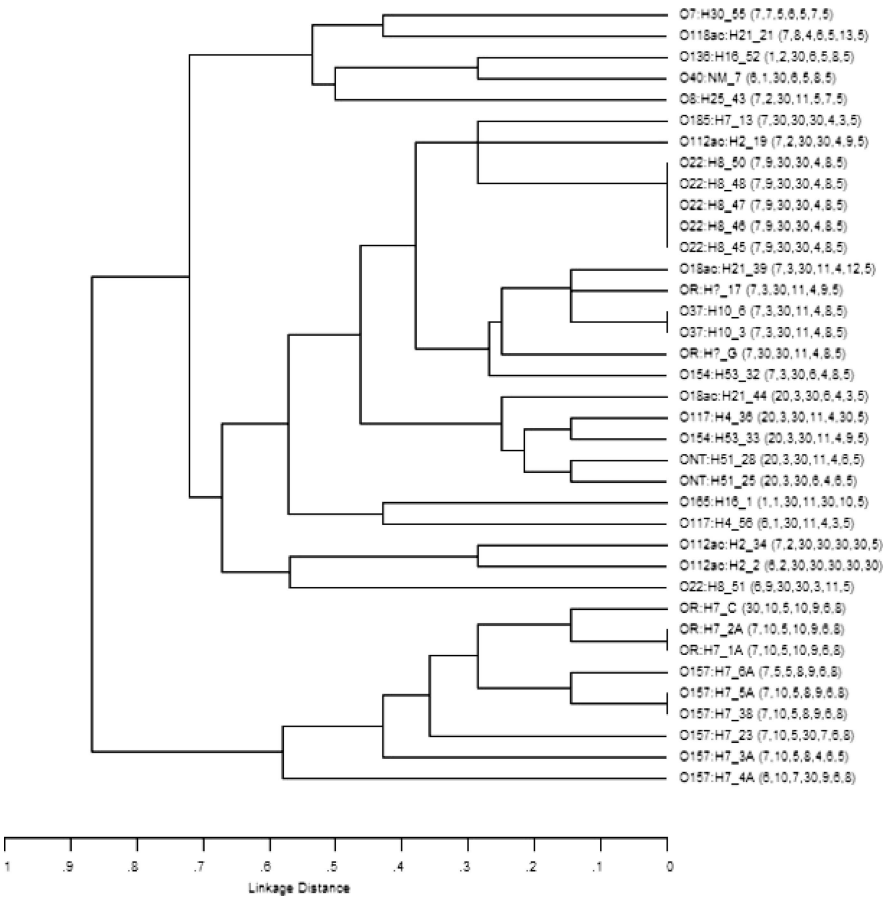

FIGURE 1. *Dendrogram based on MLVA (multiple-locus variable-number tandem repeat analysis) profiles of* Escherichia coli *isolates from cattle carcasses and feces in Mexico State slaughter plants.*

flagellin H4 antigen (O20:H4, O73:H4, and ONT:H4) *(1),* and now we can add O7:H30 and O136:H16 serotypes.

Diversity index values for CVN002, CVN004, and CVN007 were notably higher than those found previously *(3, 9).* The values calculated here for the seven VNTR loci also were higher than those reported by Amézquita-López et al. *(1).*

Although the MLVA used here is not specific for *E. coli* O157:H7, the clustering analysis highlights the genetic variability of this serotype. This high variability was previously detected using primers specific for O157 and non-O157 antigens, such as the primers used in this study *(2, 3, 38).* The OR:H7 isolates were clustered together with the O157:H7 isolates. Feng et al. *(7)* identified a rough strain of *E. coli* O157:H7 that does not produce the O side chain. The absence of the O157 antigen would make such strains undetectable or unidentifiable with most serological assays used for *E. coli* O157:H7 in clinical or food samples. Rump et al. *(35)* characterized an OR:H7 strain that had all of the genes and operons essential for O157 antigen synthesis but did not express the O157 antigen. The O rough phenotype was considered due to an IS*629* insertion in the *gne* gene (*gne*::IS*629*), which encodes an epimerase enzyme essential for the synthesis of an oligosaccharide subunit in the O antigen. Therefore, we suspect that the OR:H7 isolates analyzed here maybe actually be O157:H7 isolates with a rough phenotype.

According to the epidemiological information that was available for these cattle samples, most of the isolates that formed clusters with identical MLVA profiles probably were derived from a unique source and constitute a single clone, except for the O37:H10 isolates and some O22:H8 isolates. Same of these strains, despite having the same MLVA profile, had different virulence profiles. For the O22:H8 serotype, we suspect that isolates 45, 46, and 47 constitute one clone, and isolates 48 and 59 constitute another clone. In agreement with results of a previous study *(3),* we did not find a relationship between the presence of *vtx* genes and the MLVA profile of the isolates. Because *vtx* genes are located on temperate bacteriophages and therefore can be acquired by lateral gene transfer, this finding was not surprising.

The results of this study indicate that cattle carcasses and feces from slaughter plants in Mexico are a source of VTEC isolates that are genetically diverse in terms of serotypes and virulence profiles. Results also indicate that in addition to the *eae* colonization factor gene several of these VTEC isolates (around 42%) harbor virulence traits found among *E. coli* strains that cause serious infection in humans. The presence of these isolates in carcasses indicates the high probability that VTEC can spread during slaughter and processing. These findings reinforce the importance of continuous surveillance in cattle slaughter plants and of strategies to prevent the spread of this pathogen.

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