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Serotypes, virulence genes profiles and antimicrobial resistance patterns of *Escherichia coli* recovered from feces of healthy lambs in Mexico

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ABSTRACT

Healthy lambs are one of the major reservoirs of Shiga toxin-producing *Escherichia coli* (STEC) and it is known as the cause of foodborne diseases (FBD). The work objective is to characterize (STEC) isolates obtained from rectal swabs of healthy lambs herds, a total of 183 samples were obtained from sheep production units of the State of Mexico. *E. coli* isolates were confirmed through the amplification of the *uid A* gene. antimicrobial sensitivity pattern was determined through Kirby-Bauer (CLSI, 2012) test and the presence stx_1, stx_2 and *eae* genes from isolates by multiplex PCR. Serotyping was performed using specific anti-O and anti-H sera (SERUNAM, Mexico) for 185 Somatic and 56 flagellar antigens. 126 isolates biochemically and molecularly identified as *E. coli* were obtained, of which 80 did not express any virulence factor and 46 expressed at least some (STEC) virulence factor. The highest percentage of *E. coli* resistance was for tetracycline 48.7% (39/80), followed by nalidixic acid 13.7% (11/80), gentamicin 6.2% (5/80) and Ciprofloxacin 3.7% (3/80). Resistance to amikacin, cefotaxime and ceftazidime were not detected. A frequency of 46 STEC isolates (36.2%) were obtained, of which 28/46 (22.0%) expressed stx_1, stx_2 3/46 (6.5%), stx_1, stx_2 13/46 (10.2%) and *eae* 2/46 (1.6%). Thirty different serotypes were obtained. The three serotypes with the highest number of isolates (four each) were: O76:H19, O118:H27 and O146:H21 which have been identified as a cause of diarrhea in human population. An isolate of serogroup O104 was obtained, with a significant importance for European public health. In virtue of the discovered serotypes and the virulence factors distribution, we can affirm that the obtained isolates from lambs in the State of Mexico are classifiable as atypical STEC of low virulence.

1. Introduction

STEC strains are normal inhabitants of the ruminant gastrointestinal tract (bovine, sheep and goats) (Nataro and Kaper, 1998) and are the cause of foodborne diseases (FBDs) of animal and vegetable origin (Blanco et al., 2003; Caprioli et al., 2005).

E.coli O157:H7 has been reported in lambs and sheep; both isolated from stool, sheep meat products and byproducts (Kumar et al., 2012; Momtaz et al., 2012), on the other hand serotypes Non-O157, as OR26, O111, O103, O121, O45 and O145 have been found in calves and sheep and identified as potential public health risk factors (Evans et al., 2008; Bai et al., 2012). The most common pathotypes causing diarrhea in sheep in Mexico as EPEC (Enteropathogenic *E. coli*) and ETEC (Enterotoxigenic *E.coli*). (Méndez et al., 2013; Rangel-Vargas et al., 2015).

The phenomenon of antibiotic resistance in commensal and pathogenic bacteria has become a serious problem in public health (Saei et al., 2010), bacteriás development and persistence is a topic of global concern since these microorganisms are considered as a resistance genes reservoir, capable of transferring resistance genes to their offspring, and other organisms that cause foodborne diseases and zoonotic diseases (Zhang et al., 2002).

STEC strains can cause severe diseases in the human population as the Hemolytic-Uremic Syndrome (HUS) and Hemorrhagic Colitis (HC) (Paton and Paton, 1998a). The shiga toxins 1 and 2 and its variants are the main virulence factors of this microorganism. In addition to toxins, STEC denominated strains possess other pathogenicity mechanisms that

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allow the phenomena of attaching and effacing (A/E) as the *eae* chromosomal gene that encodes the outer membrane protein (OMP) of 94 kDa, called intimin (Paton and Paton 1998b).

Various investigations have revealed the existence of variants for both shiga toxins; the stx_1 gene has 3 subtypes (stx_{1a}, stx_{1c} and stx_{1d}) (Zhang et al., 2002; Burk et al., 2003) and numerous stx_2 gene variants have been described (stx_{2a}, stx_{2b}, stx_{2c}, stx_{2d}, stx_{2e}, stx_{2f}, and stx_{2g}) letting the stx_{2a}, stx_{2c} and stx_{2d} variants the most linked to the presentation of HUS (Piérard et al., 1998; Schmidt et al., 2000). Also exists a specific plasmid, which encodes hemolysin (ehxA) production that can contribute to the virulence of this type of microorganisms for the human population (Beutin et al., 1995; Schetuz and Strockbine, 2005).

The STEC serotype O157:H7 is commonly associated with the presence of HUS and HC in the human population, not solely more than 100 different serotypes associated with these suffering afflictions have been recognized (Nguyen and Sperandio, 2012). Its noteworthy mentioning, the STEC Non-O157 report which do not cause SHU and HC in Europe, Australia and Asia attributed to different serotypes as O5:NM (immobile), O6:H11, O26:NM, O48:H21, O91:NM, O111:NM, O113:H21, O128:H2 and O128:NM. Two of these serotypes (O11:NM and O26:H11) may also cause diarrhea to weaned calves (Goldwater and Bettelheim, 1995; Russmann et al., 1995; Achenson and Keusch, 1996).

In Mexico, few studies have been conducted to determine the presence of STEC strains and their virulence factors in cattle carcass, sheep and feces of domestic animals. Amézquita-López et al. (2014) found 12.5% prevalence of STEC, 5.4% of isolates were O157 and 7.1% of isolates were No-O157, in feces of healthy domestic animals (cattle, sheep, pigs and poultry) of small rural farms in the valley of Culiacan northwestern Mexico. Cuenca-Verde et al. (2013) found 26% prevalence of STEC No-O157 in feces of healthy sheep in one technified farm in Jalisco, Mexico. Callaway et al. (2004) found 3.3% prevalence of STEC O157:H7 isolated from pigs in the center of Mexico and Narvaez-Bravo et al. (2013) reported 23.2% prevalence of STEC O157:H7 isolated from feces of cattle and bovine animal carcasses in slaughterhouses. The objective of this research is to characterize STEC isolates and its frequency in healthy lambs in the State of Mexico.

2. Materials and methods

Sampling and bacteriological isolation

Sample size was estimated using the formula for finite populations (Wayne, 1991) with 20% *E. coli* prevalence (Mora et al., 2005). 183 rectal swabs samples were collected from healthy lambs without diarrhea of 1–6 months age during the months of February to May 2014, from eight sheep production units located in six municipalities of the State of Mexico by convenience sampling. Samples were transferred to the Center for Investigation and Advanced Studies in Animal Health of the Autonomous University of the State of Mexico (CIESA-UAEMex).

Samples were inoculated in agar Mc-Conkey (SMAC, Beckton Dickinson, USA). After 24 h of incubation at 37 °C, pink colored colonies were plated on eosin-methylene blue agar (EMB) (SMAC, Beckton Dickinson, USA) to observe the metallic luster characteristic, Finally the following biochemical tests by manitol fermentation test, TSI, LIA, MIO, H2S, indole production and presence of urease and lysine decarboxylase. Furthermore, sorbitol fermentation was tested (Fig. 1).

2.1. Genotypic identification

To corroborate isolates identity, genotypic identification through *uid A* gene amplification is proceeded in principle to extract the isolates' bacterial DNA, for which isolates were inoculated in 3 ml of BHI broth (HiMedia, USA) that were and incubated at 37 °C for 18 h. With a micropipette, 1.0 ml of culture was taken and deposited in a 1.5 mL vial subsequently centrifuged for 5 min at 9279 g (Eppendorf Centrifuge 5415D., USA), supernatant was discarded and 400 µl of distilled water were added to pellet, then vortexed during 30 s (Scientific Industries Inc. USA), then centrifuged for 5 min. at 9279 g, supernatant was discarded and 100 µl of distilled water was added, then boiled (95 °C–100 °C) during 15 min finally was slightly centrifuged and supernatant removed to be stored at −20 °C (Reyes-Rodríguez et al., 2015) until its use. For *uid A* gene amplification the following primers UAL1939 B 5′ ATGGAATTTCGCCGATTTTGC 3′ and UAL 2105 B 5′ATTGTTTGCCTCCCTGCTGC 3′ were used, with an amplification product of 187 bp previously designed and evaluated by Heijnen and Medema (2006).

2.2. Antimicrobial susceptibility test

The antimicrobial susceptibility was assessed through a disk diffusion test standardized by the National Committee for Clinical Laboratory Standards (NCCLS). *E. coli* ATCC 25922 strain was used as control bacterial inoculum of each isolate was prepared in test tubes with Mueller Hinton broth medium (MH) (SMAC, Beckton Dickinson, USA). With turbidity pattern of 0.5 at McFarland scale which approximately equivalent to $1–2 \times 10^8$ colony forming units (CFU). Ceftriaxone (30 µg), ceftazidime (30 µg), Tetracycline (30 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), amikacin (10 µg) and gentamicin (10 µg) disks were used in susceptibility test (BBL™ Sensi-Disc™ Becton Dickinson. USA).

2.3. Virulence factors

The 126 isolates that amplified *uid A* gene were analyzed by multiplex PCR, for the presence of stx_1, stx_2 and *eae*. Using the primers and conditions described by (Blanco et al., 2003) (Table 1). PCR products were visualized by electrophoresis in 2% agarose gel stained with ethidium bromide.

2.4. Serotyping

46 *E.coli* isolates were serotyped through the expression of some virulence factor and carried out according to the procedure described by Orskov and Orskov (1984). The O antisera were produced in the reference laboratory for enteric pathogens in Mexico (SERUNAM, Mexico) for 185 Somatic, and the H antisera for 56 flagellar antigens, and were obtained from the Statens Serum Institut (Copenhagen, Denmark).

3. ERIC PCR

The amplification of bacterial DNA was performed in a final volume of 50 µl containing 10 µl buffer, 6.0 µl Mg Cl2, 1.0 µl dNTPs, (dNTP Mix, Promega®, USA U151A) (200 µM of each dNTP) 1.0 µl ERIC 1 (IDT®. USA Mfg. D 173852411), 1.0 µl ERIC 2 (IDT®. USA Mfg. D 173852410), 0.5 µl Taq Polymerase, 27.5 µl free nuclease water and 3.0 µl DNA of each sample. The PCR tests were carried out Termo BIO-Rad T100 Thermal Cycler. USA, under the following conditions: initial denaturation of 5 min at 94 °C, followed by 35 cycles of 1 min at 94 °C (denaturation), 1 min at 52 °C (alignment) and 1 min at 74 °C (elongation) and final extension of 6 min at 74 °C (Soler et al., 2003).

ERIC-PCR product were displayed using 7 µl reaction product added to 3 µl loading buffer (Blue Orange 6X Loading Type PROMEGA. USA). Electrophoresis was conducted through 1.5% agarose gel in TBE Buffer (89 mM Tris, 89 mM boric acid y 2.5 mM EDTA) and ran for 60 min at 74 V. Gels were stained with ethidium bromide (0.05 mg/L) and was placed in an ultraviolet transilluminator (Mini BIS Pro Biol-Imaging Sytem Uv. USA) for their observation.

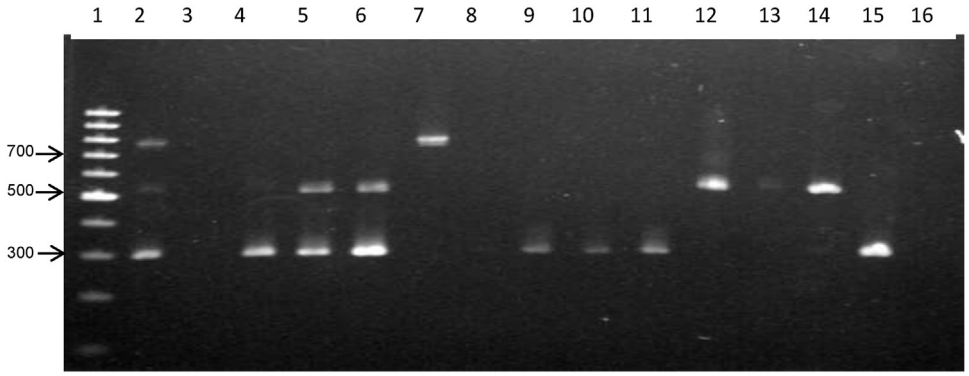

Fig. 1. Multiplex PCR band patterns of sheep STEC isolates in the State of Mexico. The *stx₁* gene 302 bp, the gene *stx₂* 516 bp and the eae gene 775 bp. Lane 1, 100 bp DNA ladder (Invitrogen™ 15628-019. 0.1 to 1.5 kb, USA). Rail 2, *E.coli* reference strain (O157:H7 EDL993). Third lane, negative control (*Corynebacterium pseudotubercolosis*), 4th (E1/4.2 stx1), 5th (E2./1.2 stx1-2), 6th (E2/C2 stx1-2), 7th (E1/9 eae), 8th (E1/4.3 negative), 9th (E1/5.2 stx1), 10th (E1/6.1 stx1), 11th (E3/C1 stx1), 12th (E1/8 stx2), 13th (E5/C1 stx2), 14th (E8/stx2), 15th (E2/6.1Stx1) and 16th (E7/C18 negative).

Table 1
Primers used for multiplex PCR.

Gene	Primer	Oligonucleotide Sequences	Fragment size (bp)	Refrence
stx₁	VT1-A	CGCTAATGTCATTCGCTCTGC	302	Blanco et al. (2003)
	VT1-B	CGTGGTATAGCTACTGTCACC		
stx₂	VT2-A	CTTCGGTATCCTATTCCCGG	516	Blanco et al. (2003)
	VT2-B	CTGCTGTGACAGTGACAAAACGC		
eae	EAE-1	GAGAATGAAATAGAAGTCGT	775	Blanco et al. (2003)
	EAE-2	GCGGTATCTTTCGCGTAATCGCC		

Statistical analysis

A variance analysis was established for antibiotics resistance results between STEC and non STEC isolates. The virulence factors results were analyzed by descriptive statistics sorting data in tables.

4. Results

Regarding a total of 183 samples of the obtained rectal swabs, 126 isolates were confirmed by the amplification of the *uid A* gene, which determines an *E. coli* isolate frequency of 68.8%. (Table 2). Of 126 isolates, 46 expressed at least one of the studied virulence factors and were serotyped, while 80 did not express any of the studied virulence factors both isolates that expressed some or any virulence factor were subjected to in vitro sensitivity test ($p > 0.05$). (Table 3).

Concerning *E. coli* antimicrobial resistance, the greatest resistance percentage was observed for tetracycline (TE) with 48.7% (39/80) followed by the nalidixic acid (NA) with 13.7% (11/80), gentamicin (GE) 6.2% (5/80), ciprofloxacin (CIP) 3.7% (3/80). Amikacin ceftriaxone and ceftazidime did not present any resistance.

Table 2
E.coli isolates no.

UPP	Municipal	Sample No.	*E.coli* BQ	*uidA*
1	Xalatlaco	20	19/95%	16/80%
2	Ocoyoacac	22	20/90.9%	20/90.9%
3	Calimaya	20	15/75%	13/65%
4	Metepec	16	15/93.7%	15/93.7%
5	Temoaya	25	16/64%	16/64%
6	Texcalyacac	21	7/33.3%	7/33.3%
7	Jiquipilco	36	27/75%	27/75%
8	Ocoyoacac	23	12/53.1%	12/53.1%
		183	131/71.5%	126/68.8%

Table 3
E.coli isolates antibiotic resistance without any virulence factor expression and STEC isolates.

	E.coli isolates resistance without any virulence factor expression		STEC isolates resistance	
Antibiotic	No.	%	No.	%
TE	39/80	48.7	12/17	70.7
NA	11/80	13.7	8/17	47
GE	5/80	6.2	2/17	11.7
CIP	3/80	3.7	0/17	0
AM	0/80	0	2/17	11.7
CAZ	0/80	0	0/17	0
CTX	0/80	0	0/17	0

$P > 0.05$ (No significant differences).

In 17/46 STEC isolates that expressed phenotypic resistance, tetracycline was the antibiotic with the largest number of resistant strains with 70.7% (12/17), nalidixic acid 47.7% (8/17), gentamicin 11.7% (2/17), amikacin 11.7% (2/17), ciprofloxacin, ceftazidime and cefotaxime did not presented any resistance (Table 3). It was observed that 6 STEC isolates with different serotypes and virulence genes expression profile, expressed phenotypic resistance to more than one antibiotic (Table 4).

46 isolates expressed some virulence genes 36.5% (126/46), of which: 28 (22.2%) expressed *stx₁*, 3 expressed *stx₂* (2.3%), 13 expressed both *stx₁* and *stx₂* (10.3%) and two isolates expressed *eae* (1.5%) (Table 4).

STEC isolates belonged to 19 different serogroups O and 30 different serotypes O:H. 68% of the isolates belonged to one of the following five serogroups: O8, O76, O118,O146, O153, O176, O185 and O187, 39.1% of the isolates belonged to the following serotypes: O76:H19, O118:H27, O146:H21, O176:NM and O187:H28. (Table 4)

Forty percent of the isolates belonged to O79:H19, O118:H27 and O146:H21 serotypes (4 isolates/serotype). The 20% of the isolates belonged to O176:NM and O187:H21 serotypes(3 isolates/serotype) (Table 4).

Its noteworthy mentioning the presence of O104:H7, O146:H21, O76:H19, and ONT:H21 serotypes causing diarrhea in humans. (Table 4)

ERIC-PCR was realized to evaluate the clonal relationship between *E. coli* isolates that expressed some virulence factor, 44/46 STEC isolates showed a 95.6% of genetic diversity. Only two isolates obtained from male lambs, of the same production unit were sensitive to all used antibiotics and showed the same bands pattern through the expression of *stx₁* gene, which could mean a same clonal profile. Only varied in serotype expression, one expressed O176:H19 serotype and the other

Table 4
STEC isolates serotypes and virulence factors of isolates obtained from healthy sheep feces.

Serotype	Isolates	isolates No	Resistance profile	Virulence factores.			
				stx_1	stx_2	stx_1 and $_2$	*eae*
O2:NM	E8 C13	1	NA, TE		X		
O8:H9	E5 C1	1	S		X		
O8:H20	E1 4.2	1	GM,TE	X			
O8:H49	E2 5.1	1	S	X			
O17:H18	E1 2.1	1	S			X	
O43:H2	E7 C18	1	NA,GM,TE	X			
O55:H19	E7 C17	1	TE	X			
O65:H38	E1 3.1	1	NA,TE			X	
O76:H19	E2 9.2	4	S	X			
O76:H19	E7 C19		S	X			
O76:H19	E3 M3		S	X			
O76:H19	E3 M7		S	X			
O76:NM	E2 4.2	2	S	X			
O76:NM	E7 C1		S	X			
O104:H7	E5 C10	1	NA,TE	X			
O118:H27	E1 5.1	4	S	X			
O118:H27	E1 9		S				X
O118:H27	E2 2.2		S			X	
O118:H27	E1 8		S		X		
O118:H41	E1 6.1	1	S	X			
O128 AC:NM	E5 C11	1	S			X	
O139:NM	E3 C5	1	AM	X			
O146:H21	E2 5.2	4	S			X	
O146:H21	E4 C13		S			X	
O146:H21	E2 4.1		NA			X	
O146:H21	E2 1.2		NA			X	
O153:H21	E3 C10	2	AM	X			
O153:H21	E3 M6		S	X			
O154:H21	E2 6.2	1	TE	X			
O174:H8	E4 C19	1	TE			X	
O176:NM	E6 C15	3	S	X			
O176:NM	E8 C19		S	X			
O176:NM	E4 C18		S			X	
O176:H9	E 6 C11	1	S	X			
O176:H19	E8 C6	2	S	X			
O176:H19	E 8 C16		S	X			
O179:H8	E2 9.1	1	TE				X
O185:NM	E4 C8	1	TE			X	
O185:H10	E1 5.2	1	NA,TE	X			
O185:H19	E7 C28	1	NA	X			
O187:H28	E3 M10	3	S			X	
O187:H28	E4 C11		S			X	
O187:H28	E3 M10		S	X			
H7	E3 C4	1	TE	X			
H21	E3 C10	1	S	X			
O H	E3 M5	1	S	X			

Abbreviations: Resistant to: TE: Tetracycline, GE: gentamycin, AM: Amikacin and NA: Nalidixic Acid. S: sensitive to antibiotics used: Tetracycline, Gentamicin, amikacin, nalidixic acid, ciprofloxacin, ceftazidime and ceftriaxone.

O176:H̄ serotype (Fig. 2)

5. Discussion

The constant monitoring of the antibiotics used in animal husbandry is relevant to determine the presence and prevalence of resistant strains that potentially can be a risk factor for human health (Schroeder et al., 2002).

With regard to the *E.coli* isolates antibiotics resistance, in this research we found a resistance to tetracycline of 48.7%, followed by nalidixic acid 13.7%, gentamicin 6.2% and finally ciprofloxacin with 3.7%, amikacin, ceftazidime and ceftriaxone did not present phenotypic resistance. In Ontario province, Canada, 2012, reported 12% resistance to tetracycline and 100% sensitivity to ciprofloxacin and amikacin and nalidixic acid for *E. coli* isolates from sheep flocks (Scott et al., 2012). Scott, relates the resistance level of tetracycline to its inappropriate use in diets and drinking water that could be a risk factor to animal and human population.

In Argentina Pantozzi et al. (2010) found in sheep *E.coli* isolates with a resistance of 21.0% to tetracycline, with similar reports for ceftriaxone of 5.3%. Regarding gentamicin, amikacin, ciprofloxacin and nalidixic acid, no resistance was reported. Novotna et al. (2005) in Jordan, single strain resistant to tetracycline was reported, with a percent well below the 39 isolates reported in the present investigation under different geographic and productive conditions.

In Spain sheep *E.coli* isolates, a resistance of 26.3% to nalidixic acid a little higher than what reported in the present work. (Orden et al., 2000). Blanco et al. (1996) in VTEC sheep isolates (Extremadura region, Spain), where the climatic conditions are different presented resistance percentages to tetracycline 76.0% higher than what reported in our study, although it remains the antibiotic with the highest resistance percentage. Respecting gentamicin, the percent was similar to what estimated in this study, and for nalidixic acid, Blanco reported 6%, a lower percent than the reported in this study.

In this research, STEC isolates were reported with phenotypic expression resistant to tetracycline 70.7%, nalidixic acid 47.1%, gentamicin 11.7%, amikacin 11.7%, regarding ciprofloxacin, ceftriaxone and ceftazidime no phenotypic resistance was reported. In addition, a phenotypic expression resistance was observable for more than one antibiotic, where four isolates expressed resistance to tetracycline-nalidixic acid, one isolate to tetracycline-gentamicin and one isolate to tetracycline-gentamicin-nalidixic acid.

A work realized in Brazil with sheep STEC isolates of Dorper race, in tropical conditions (Ferreira et al., 2015) reported a lower percent for gentamicin 4.5% and highest resistance for tetracycline antibiotic with 12.2%, a percent lower than the reported in this research. Ferreira et al. (2015) were able to detect resistance to ceftazidime with a 5.6%, in comparison with the present study where no cephalosporin resistance was reported, lastly, Ferreira et al. (2015) reported a 1.1% resistance for amikacin.

In an investigation carried out in the Valley of Culiacán, Sinaloa with healthy backyard sheep Amézquita-López et al., (2016) reported STEC isolates, with resistance to amikacin, gentamicin and tetracycline, also reported isolates with serotype O146:H21 resistant to cephalosporin, in the present work we obtained the same serotype but with resistance to nalidixic acid. It is important to mention that Amézquita-López et al. (2016) reported in Mexico resistance to more than one antibiotic cephalothin-chloramphenicol, cephalothin-Gentamicin, Ampicillin-cephalothin-tetracycline, ampicillin-cephalothin-chloramphenicol-tetracycline in STEC O157 and No-O157 isolates.

In the USA (Edrington et al., 2009) reported resistance to more than one antibiotic in O157:H7 isolates obtained from lambs, reported two isolates resistant to two antibiotics and an insolate resistant to eight antibiotics.

In the present investigation we were able to find a 36.5% (46/126) of STEC isolates. The resistance percentage was similar to the one reported in India, 32% of isolates obtained from lambs under a transhumance system (Bandyopadhyay et al., 2011), in Iran 36.8% (Tahamtman and Namavari et al., 2014), in Spain 35% in healthy lambs (Rey et al., 2003), in Australia 31% (Djordjevic et al., 2001) and in New Zealand 48% of STEC in lambs (Cookson et al., 2006).

There are reports of STEC slightly lower than that reported in this study, in Mexico 26% from healthy sheeps (Cuenca-Verde et al., 2013). In India reported 24.1% of STEC presentation in lambs with and without diarrhea (Wani et al., 2009), also in India, 24% found in lamb's samples (Kumar et al., 2012). In Iran, a research established with healthy sheep 22.4% (Ghanbarpour and Kiani, 2013) and in China reported a STEC percent bit less of 19.8% in healthy sheep (Gu et al., 2011).

In this investigation stx_1 gene was reported as the virulence factor with the highest presentation frequency of 22.2%, followed by stx_1-stx_2

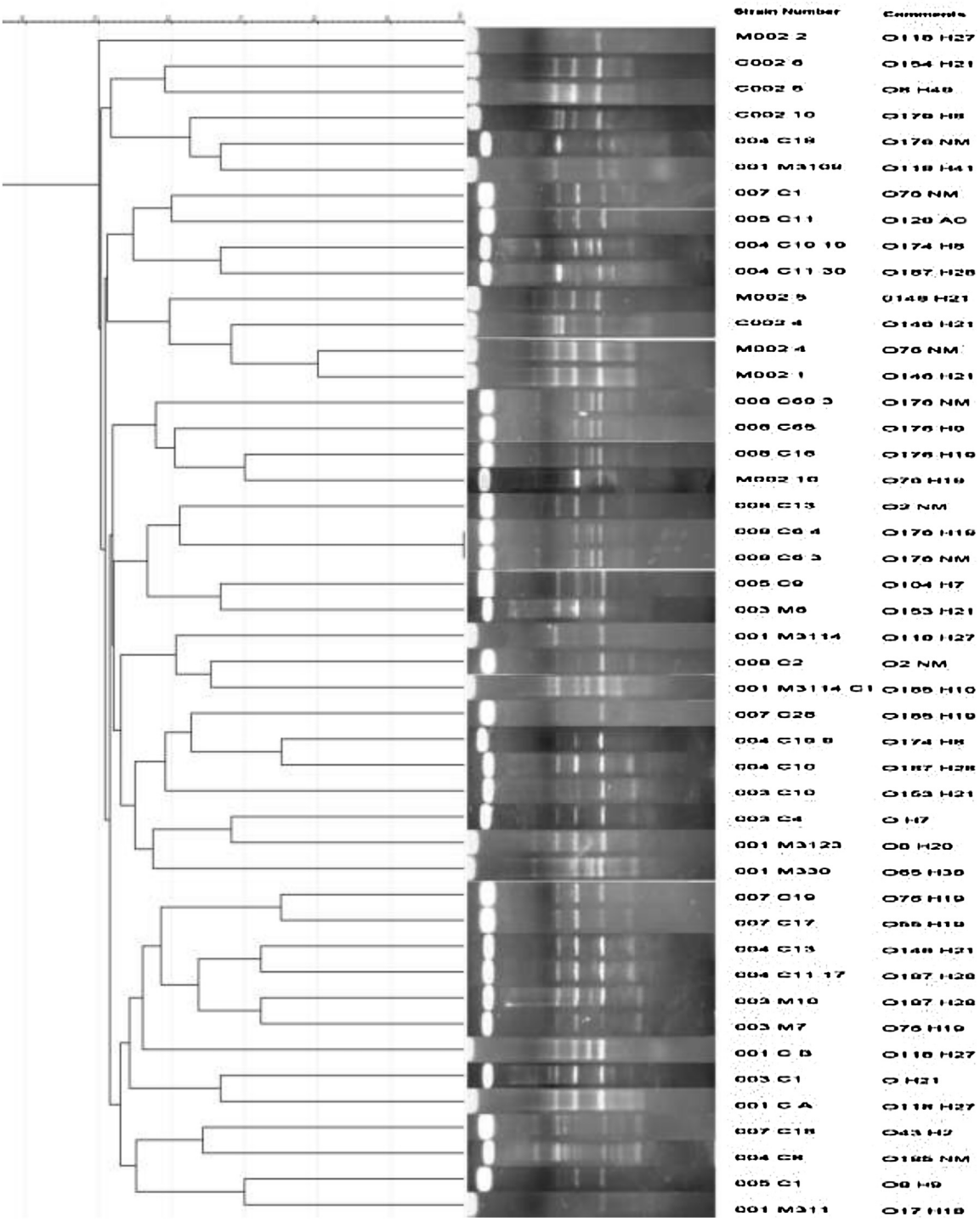

Fig. 2. ERIC-PCR bands pattern dendrogram of 46 *E coli* STEC isolates. 44 presented different band patterns and two presented similar band patterns.

10.3%, stx_2 2.3%, regarding *eae* gene this paper shows a frequency of 1.5%. There are different investigations carried out for STEC detection around the world which is also considered as the distribution pattern of the virulence stx_1, stx_2 and *eae* genes, which coincide with the present study (Blanco et al., 2003; Brett et al., 2003; Zweifel et al., 2004; Wani et al., 2009; Cookson et al., 2006; Cuenca-Verde et al., 2013; Tahatman and Namavari, 2014; Ferreira et al., 2015) where stx_1 presented the greater virulence factor percentage of all isolates followed by $stx_{1\text{-}2}$, then stx_2 and finally *eae*. In case of *eae* gene, this study reported similar results to those reported in Switzerland (Zweifel et al., 2001), Spain (Rey et al., 2003) and India (Bhat et al., 2008).

These data suggest that the reported isolates when not expressing the stx_1, stx_2 and *eae* genes together, could be named atypical STEC and thus of low virulence, however, continue representing a risk factor to public health, since isolates have been reported lacking the expression of *eae* gene as a cause of diarrhea in humans (Strockbine et al., 1997; Nataro and Kaper 1998; Kumar et al., 2004).

In this research, an isolate of serotype O104:H7 with expression of stx_1 gene was detected, belonging to O104 serogroup. This serogroup gained more importance since it introduced a severe outbreak of hemolytic uremic syndrome in Germany in 2011 attributed to O104:H4 serotype present in products with plant origin, possibly water was contaminated with residues of ruminant's feces (Mora et al., 2011; Duffy et al., 2014).

Another diarrhea causing STEC serotype in human population is O146:H21, in this investigation four isolates are reported with expression of stx_1 and stx_2 genes. Researches in other countries reported this same serotype from healthy adult sheep and lambs, in Spain (Blanco et al., 2003; Rey et al., 2003; Mora et al., 2005); Mexico (Cuenca-Verde et al., 2013 Amézquita-López et al., 2014), Brazil (Vettorato et al., 2009) and Norway (Urdahl et al., 2001) isolates from healthy adult sheep and lambs. Serotypes O104:H7 and O146:H21 along with O76:H19 serotype (with expression of stx_1 gene) and ONT:H21 (with expression of stx_1 gene) found in this study are responsible for cases of diarrhea in humans in Mexico; which makes these serotypes, in particular, a possible risk factor for public health (Eslava et al., 1994).

With regard to ERIC-PCR study, 44 of the 46 isolates showed different bands patterns, only two isolates showed similar bands patterns, which suggest a same clonal profile; these two isolates possess the same resistance pattern to the used antibiotics, expressing stx_1 gene and belonged to the same serogroup, differ only in the flagellar H antigen expression, the isolate 008 C16 express O176:H19 serotype, while 008 C2 isolate express O176:H̄ serotype. This can be explained by the variation in H antigen expression of the phase variation phenomenon (Stainer et al., 1994), which explains that some *E. coli* isolates have two genetic determinants sets for different flagellar antigens, susceptible to alternation regarding its phenotypic expression, as bacteria multiply. Presenting with a certain probability, the variant *fliC* H19 flagellar gene is responsible for this variation, because it is responsible for proteins polymerization, forming bacterial flagellar filaments (Osek and Gallien, 2002).

6. Conclusions

The STEC isolates frequency was 36.5% (46/126), no significant statistically difference was observed for antibiotics resistance among *E.coli* isolates and STEC. It was not able to detect isolates with the stx_1, stx_2 and *eae* genes expression simultaneously, through which STEC isolates fell into low virulence atypical STEC category. STEC isolates with serotypes that cause diarrhea in humans were detected with importance to Mexican public health, like O146:H21, O104:H7, O76:H19 and ONT:H21.

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