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Serotypes, Pathotypes, Shiga Toxin Variants and Antimicrobial Resistance in Diarrheagenic *Escherichia coli* Isolated from Rectal Swabs and Sheep Carcasses in an Abattoir in Mexico

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Abstract: Sheep represent one of the main reservoirs of diarrheagenic *Escherichia coli*; this microorganism is an etiological agent of food-borne diseases; therefore, this work aimed to identify and characterize the principal pathotypes of diarrheagenic *E. coli* (DEC) obtained through rectal swabs and carcasses samples from sheep slaughtered in an abattoir at the central region of Mexico. The isolates were subjected to bacteriological identification, serotyping; phylogenetic classification; detection for virulence factors, and antimicrobial sensibility. A total of 90 *E. coli* isolates were obtained. It was observed through 49 *E. coli* isolates (54%), 8 of them from carcasses, and 43 from feces was DEC. DEC serotypes with health public relevance were found: O76:H19 (n = 5), O146:H21 (n = 3), O91:H10 (n = 1), O6:NM (n = 1), and O8:NM (n = 1). Regarding the presence of Shiga toxin-producing *E.coli* (STEC), 43/90 (47.7%) isolates have the *stx1* w/o *stx2* genes, and therefore were assigned as STEC non-O157; only one isolate expressed *stx1* and *eae* genes and was classified as t-STEC (typical STEC). Additionally, 3/90 (3.3%) harbored only the *eae* gene and were classified as enteropathogenic *E. coli* (EPEC), the *stp* gene was found in 2/90 isolates (2.2%) and were classified as enterotoxigenic *E. coli* (ETEC); 1/90 (1.1%) isolates harboring the *ipaH* were classified as enteroinvasive *E. coli* EIEC. Regarding *stx1* genes subtypes, *stx1c* only was found in 60.5% (26/43), followed by *stx1a-stx1c* 20.9% (9/43) and *stx1a-stx1d* 2.3% (1/43). The presence of both, *stx1* and *stx2* genes was found in 7/43 isolates (16.3%) from rectal swabs; the combination *stx1c-stx2g* was detected in 3/43 isolates (6.9%), while 4 (9.4%) isolates showed different patterns (*stx1a-stx1c-stx2g*; *stx1c-stx2b-stx2g*; *stx1c-stx2b* and *stx1a-stx1c-stx2b-stx2g*). STEC isolates showed the major diversity of phylogenetic groups, although phylogroup B1 was predominant in 90.6% (39/43) while there was only one isolate (2.3%) in each remaining phylogroup (A, B2, C, and F). All EPEC, ETEC, and EIEC isolates were clustered in phylogroup B1. We observed that 27.9% (12/43) of STEC isolates carried at least one antibiotic resistance: nine isolates expressed the *tetB* gene, one isolate the *tetA* gene, two isolates the *sul2* gene, one isolate the *sul1* and one isolate the *sul1-tetB* genes. These results highlight the importance of diarrheagenic *E. coli* as a potential risk for public health during the slaughtering process.

Keywords: diarrheagenic *Escherichia coli*; serotyping; pathotypes; phylogroup; sheep; antibiotic resistance; slaughterhouse

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1. Introduction

Sheep and other ruminants are regular carriers of commensal *Escherichia coli*; however, they may harbor some pathogenic *E. coli* and cause either, diarrhea or extraintestinal illness [1]. The relevance of these diarrheagenic *E. coli* (DEC) isolates as causative agents of

food-borne diseases (FBD) was recently studied in Latin America, although there is a lack of information in some countries regarding the main reservoirs and infection routes [2]. Animal products, like sheep and beef meat, are at risk of contamination by poor hygiene practices during the slaughtering process in the abattoirs, hence, the implementation of good production practices (GPP) and good manufacture practices (GMP) are essential to prevent bacterial contamination of carcasses and ensure food safety [3]. Sheep without diarrhea are usually asymptomatic carriers of zoonotic pathogens and reservoirs of DEC, which could enter the production line, especially in the critical control points [4]. The animals that arrive at the slaughterhouse are the principal focus of contamination towards drinking water and animal products, allowing the direct transmission of zoonotic microorganisms to the human population [5].

At least five *E. coli* pathotypes are related to gastrointestinal illness in humans: Shiga-toxin-producing *E. coli* (STEC), enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), and enteroaggregative *E. coli* (EAEC) [6]. These pathotypes are classified according to their virulence factors. The principal virulence factor of STEC is the production of a toxin that inhibits protein synthesis coded by *stx1* and *stx2* genes and their variants; moreover, other virulence factors like the intimin (encoded by *eae* gene) or autoagglutinating adhesins can be found [7].

Shiga toxins are classified as *stx1* and *stx2*. The *stx1* toxins are a homogeneous group with three subtypes: *stx1a*, *stx1c*, and *stx1d*. On the other hand, *stx2* toxins are more heterogeneous with a greater number of subtypes that include *stx2a*, *stx2b*, *stx2c*, *stx2d*, *stx2e*, *stx2f* and *stx2g*, with *stx2c* and *stx2d* being the most strongly associated with hemolytic uremic syndrome (HUS). Other relevant virulence factors include the intimin (encoded by *eae* gene), a plasmid-carried enterohaemolysin (encoded by *ehxA* gene), and putative adhesins genes like *Tox B*, *saa*, *espC*, and *espP* [8,9].

The presence or absence of the *eae* gene in STEC strains allows classifying them into the typical virulent (t-STEC) or atypical strains of low virulence (a-STEC). STEC strains induce gastroenteritis and further complications such as HUS or hemorrhagic colitis (HC), which can lead to chronic kidney dysfunction, especially in infants and the elderly [6]. EPEC produces the attachment and effacing (A/E) lesions onto intestinal mucosa. This pathotype is divided into two categories based on the presence or absence of the bundle-forming pilus (*bfp*) gene; strains that contain this gene are classified as typical (t-EPEC), while the ones that lack this gene are atypical (a-EPEC). Curiously, the a-EPEC strains are more common in developing countries; in contrast, t-EPEC causes diarrhea in children from developed countries [10]. The main feature of ETEC is the production of two enterotoxins: the heat labile-toxin (LT) and the heat-stable toxin (ST). The ETEC strains are the leading cause of traveler's diarrhea and also related to children's diarrhea [11].

The EIEC group and *Shigella* spp. are biochemically and genetically related. The pathogenicity mechanism is through the invasion of the colon´s epithelium; several involved proteins like Ipa and others are encoded in the 140 MDa plasmid *pInv*. Generally, watery diarrhea is observed, but in some cases, inflammatory colitis can occur [12]. Finally, EAEC strains are characterized by aggregative adherence (AA) to Hep-2 cells, wherein bacteria are seen in stacked brick aggregates attaching to cells. Adherence is due to aggregative adherence fimbriae encoded by the *aggA* gene, especially in variant I (AAF/I) [13].

Several studies have reported DEC in carcasses from slaughterhouses; for example in Burkina Faso, the five pathotypes mentioned in this work were isolated from bovine, poultry, and swine carcasses [14]. France UM et al. (2018) [15] reported the presence of STEC and EPEC in bovines. In Mexico there are few reports about these pathotypes in ruminant carcasses [16]; such information is necessary to assert the risk factors that could affect the safety of sheep carcasses in this country. We investigated the prevalence of DEC isolates obtained from sheep slaughtered in an abattoir in Mexico, and determined the presence of virulence factors, the phylogenetic classification of isolates as well as their antimicrobial resistance profile. Therefore, the main objective of this work is to know which diarrheal pathotypes of *E. coli* are naturally present in sheep slaughtered in a slaughterhouse in

the state of Mexico and to identify if they could represent a risk factor for the consuming population.

2. Materials and Methods

2.1. Sample Collection and Bacteriological Isolation

A convenience sampling was performed in a slaughterhouse with the largest number of slaughtered sheep in the central region of Mexico. The sample size was estimated with a prevalence of 12.3% [17] and a 95% confidence level through sample size determination for finite populations [18]. A non-destructive method employing a swab in 0.1% peptone + NaCl (0.85%), according to the European Union, was used [19]. From a total of 321 samples, 159 rectal swabs were taken before evisceration and 162 swab samples were taken from carcasses after final washing and before refrigeration. Finally, swabs were stored in sterile tubes with 25 mL of peptone water (1%).

Samples were transported to Centro de Investigación y Estudios Avanzados en Salud Animal (CIESA, Universidad Autónoma del Estado de México). Samples were streaked onto MacConkey Agar (MAC, Beckton Dickinson, Franklin Lakes, NJ, USA). After 24 h of incubation at 37 °C, suspected pink colonies were grown in Eosin Methylene Blue Agar (EMB, Dickinson, Franklin Lakes, NJ, USA), and colonies with a green metallic sheen were identified by biochemical tests (triple sugar iron, sulfide indole motility, methyl-red Voges-Proskauer, urea, malonate, phenylalanine, gluconate, citrate, and sorbitol) [20].

2.2. Serotyping

The procedure described by Orskov and Orskov (1984) [21] was employed. Specific rabbit sera against 187 *E. coli* somatic (O) antigens and 53 flagellar (H) antigens were used (SERUNAM, registered trademark in Mexico, with number 323158/2015).

2.3. Phylogenetic Group Determination

A quadruplex PCR was carried out to identify the phylogenetic groups (A, B1, B2, C, D, E, and F), the *chuA*, *yjaA*, *arpA*, and *TspE4.C2* genes were amplified with primers and PCR conditions according to Clermont et al. (2013) [22] (Table 1).

2.4. Virulence Factors

The identification and characterization of diarrheagenic *E. coli* pathotypes (STEC, EPEC, ETEC, EIEC, and EAEC) were performed by PCR. Fragments of several virulence genes were amplified and assigned to each pathotype employing primers and thermal cycling conditions, as described previously [11,23–27] (Table 1). The reaction products were visualized on 2% agarose containing ethidium bromide.

Table 1. Primers used in phylogenetic group determination and virulence factors identification.

Gene or Probe	Description of Target	Oligonucleotide Sequence (5′–3′)	PCR Product (pb)	Reference
vtx1	Verocytotoxin type 1	GTACGGGGATGCAGATAAATCGC AGCAGTCATTACATAAGAACGYCCACT	209	[27]
vtx2	Verocytotoxin type 2	GGCACTGTCTGAAACTGCTCCTGT ATTAAACTGCACTTCAGCAAATCC	627	[27]
		CGCTGTCTGAGGCATCTCCGCT TAAACTTCACCTGGGCAAAGCC	625	
eae	Intimin	TCAATGCAGTTCCGTTATCAGTT GTAAAGTCCGTTACCCCAACCTG	482	[25]
Bfp	Bundle-forming pilus	AATGGTGCTTGCGCTTGCTGC GCCGCTTTATCCAACCTGGTA	300	[23]

Table 1. *Cont.*

Gene or Probe	Description of Target	Oligonucleotide Sequence (5′–3′)	PCR Product (pb)	Reference
LT	Heat-labile toxins	ACGGCGTTACTATCCTCTC TGGTCTCGGTCAGATATGTG	273	[11]
STp	Heat-stable toxins	TCTTTCCCCTCTTTTAGTCAG ACAGGCAGGATTACAACAAAG	166	[11]
ipaH	Invasion plasmid antigen	TGGAAAAACTCAGTGCCTCT CCAGTCCGTAAATTCATTCT	423	[26]
aggR	Transcriptional activator of AAFs	CTAATTGTACAATCGATGTA ATGAAGTAATTCTTGAAT	308	[24]
chuA	Outer membrane hemin receptor ChuA	ATGGTACCGGACGAACCAAC TGCCGCCAGTACCAAAGACA	288	[22]
yjaA	Uncharacterized protein YjaA	CAAACGTGAAGTGTCAGGAG AATGCGTTCCTCAACCTGTG	211	[22]
TspE4.C2	Putative gene for a lipase	CACTATTCGTAAGGTCATCC AGTTTATCGCTGCGGGTCGC	152	[22]
arpA	Ankyrin repeat protein A	AACGCTATTCGCCAGCTTGC TCTCCCCATACCGTACGCTA	400	[22]

2.5. Detection of Shiga Toxin Subtypes

Identification of *stx1* and *stx2* subtypes genes (*stx1a*, *stx1c*, *stx1d*, *stx2a*, *stx2b*, *stx2c stx2d*, *stx2e*, *stx2f*, *and stx2g*) was carried out with primers and PCR conditions described by Scheutz et al. (2012) [27]. Amplicons were visualized on a 2% agarose gel with ethidium bromide (Table 2).

Table 2. Primers used in the identification of variants of Shiga toxin.

Gene or Probe		Oligonucleotide Sequence (5′–3′)	PCR Product (pb)	Reference
vtx1a	*vtx1a-F1* *vtx1a-R2*	CCTTTCCAGGTACAACAGCGGTT GGAAACTCATCAGATGCCATTCTGG	478	[27]
vtx1c	*vtx1c-F1* *vtx1c-R1*	CCTTTCCTGGTACAACTGCGGTT CAAGTGTTGTACGAAATCCCCTCTGA	252	[27]
vtx1d	*vtx1d-F1* *vtx1d-R1*	CAGTTAATGCGATTGCTAAGGAGTTTACC CTCTTCCTCTGGTTCTAACCCCATGATA	203	[27]
vtx2a	*vtx2a-F2* *vtx2a-R3*	GCGATACTGRGBACTGTGGCC CCGKCAACCTTCACTGTAAATGTG	349	[27]
vtx2b	*vtx2a-R2* *vtx2b-F1* *vtx2b-R1*	GGCCACCTTCACTGTGAATGTG AAATATGAAGAAGATATTTGTAGCGGC CAGCAAATCCTGAACCTGACG	347 251	[27]
vtx2c	*vtx2c-F1* *vtx2c-R2*	GAAAGTCACAGTTTTTATATACAACGGGTA CCGGCCACYTTTACTGTGAATGTA	177	[27]
vtx2d	*vtx2d-F1* *vtx2d-R1* *vtx2d-R2*	AAARTCACAGTCTTTATATACAACGGGTG TTYCCGGCCACTTTTACTGTG GCCTGATGCACAGGTACTGGAC	179 280	[27]
vtx2e	*vtx2e-F1* *vtx2e-R2*	CGGAGTATCGGGGAGAGGC CTTCCTGACACCTTCACAGTAAAGGT	411	[27]
vtx2f	*vtx2f-F1* *vtx2f-R1*	TGGGCGTCATTCACTGGTTG TAATGGCCGCCCTGTCTCC	424	[27]

2.6. Antimicrobial Susceptibility Testing

Susceptibility to antibiotics was tested using a disk diffusion method according to Clinical and Laboratory Standard Institute guidelines [28]. *E. coli* ATCC 25922 and ATCC 35218 were used as quality control. Commercial discs of ampicillin 10 µg (AMP), cephalothin 30 µg (CEF), ceftazidime 30 µg (CAZ), amikacin 30 µg (AMK), ciprofloxacin 5 µg (CIP), gentamicin 10 µg (GEN), fosfomycin 50 µg (FOF), netilmicin 30 µg (NET), trimethoprim-sulfamethoxazole 25 µg (SXT), norfloxacin 10 µg (NOR), nitrofurantoin 300 µg (NIT), and tetracycline 30 µg (TET) (BBL™Sensi-Disc™Becton Dickinson, Franklin Lakes, NJ, USA) were used.

2.7. Antimicrobial Resistance Genes

To identify antimicrobial resistance genes against β-lactams, tetracyclines, and sulfonamides, the genes *bla*TEM, *tetA*, *tetB*, *sul1*, and *sul2* were analyzed by the PCR technique using the primers and conditions described by Kerrn et al. 2002 [29], Martí et al. 2006 [30] and Dallenne et al. 2010 [31], respectively (Table 3). The PCR products were visualized by electrophoresis on a 2% agarose gel stained with ethidium bromide.

Table 3. Primers used in the identification of resistance genes.

Gene or Probe	Description of Target	Oligonucleotide Sequence (5′–3′)	PCR Product (pb)	Reference
sul1	*sul1 F* *sul1 R*	CGG CGT GGG CTA CCT GAA CG GCC GAT CGC GTG AAG TTC CG 3	433 pb	[29]
sul2	*sul2 F* *sul2 R*	GCG CTC AAG GCA GAT GGC ATT GCG TTT GAT ACC GGC ACC CGT	293 pb	[29]
tetA	*tet A F* *tet A R*	GTA ATT CTG AGC ACT GTC GC CTG CCT GGA CAACAT TGC TT	950 pb	[30]
tet B	*tet B F* *tet B R*	GTT AGG GGC AAG TTT TG GTA ATG GGC CAA TAA CAC CG	650 pb	[30]
BlaTEM	*MultiTSO-T BlaTEM F* *MultiTSO-T BlaTEM R*	CAT TTC CGT GTC GCC CTT ATT C CGT TCA TCC ATA GTT GCC TGA C	800 pb	[31]

3. Results

3.1. Bacterial Isolation

Overall, 321 samples were collected: 159 from rectal swabs and 162 from carcasses. A total of 90 *E. coli* isolates were obtained and confirmed by biochemical test and serotyping, 15 of them from carcasses, and 75 from feces, providing a frequency of 28%. Out of 49 *E. coli* isolates (54%), 8 of them were from carcasses, and 43 from feces, and these expressed at least one virulence factor included in this study. The remaining 41 isolates (46%) did not express any virulence factor.

3.2. Serotyping

Serotyping results showed that STEC pathotype gathered 23 different O serogroups and 33 serotypes O:H. The most frequent serogroups were O76 and O146 (11.6%), followed by serogroups O176 and O91 (6.9%). The EPEC, ETEC, and EIEC pathotypes were distributed in six different serotypes O:H. DEC serotypes with public health relevance were found: O76:H19 (n = 5), O146:H21 (n = 3), O91:H10 (n = 1), O6:NM (n = 1), and O8:NM (n = 1) (Table 4).

Table 4. Association between serotype, phylogenetic group and virulence genes of diarrheagenic *E. coli* (DEC) isolated from sheep slaughtered in an abattoir.

Isolate	Serotype	Source	Virulence Factor					PG
			***stx*$_1$ Variants**	***stx*$_2$ Variants**	***eae***	***stp***	***Ipah***	
E44	O53:H51	rectal	1c					A
* Z15	-:H10	rectal			+			B1
C28B	-:H14	rectal	1c					B1
* D3	-:H34	carcass			+			B1
Z2	-:H34	rectal	1c		+			B1
Z29	-:H16	rectal	1a1c					B1
V25	O100:H21	rectal	1c					B1
V22	O100:H28	rectal	1c	2g				B1
D28	O104:H2	carcass	1c					B1
# D15	O105 AB:H16	carcass				+		B1
# D15B	O120:H16	carcass				+		B1
V13	O146:H10	rectal	1c					B1
C23	O146:H21	rectal	1c	2b–2g				B1
Z3	O146:H21	rectal	1c					B1
Z19	O146:H21	rectal	1c					B1
B7	O146:H8	rectal	1a–1c	2g				B1
B13C	O150:NM	carcass	1c					B1
Z5	O174:H16	rectal	1c					B1
Z25	O174:H16	rectal	1a–1c					B1
V15	O176:NM	rectal	1c					B1
C24	O185:NM	rectal	1c	2b				B1
* D53	O28 AC:H21	carcass			+			B1
° E3	O28 AC:H21	rectal					+	B1
E15B	O32:H27	rectal	1c					B1
E20	O32:H7	rectal	1c					B1
E30	O32:H7	rectal	1c					B1
D26	O34:H14	carcass	1c					B1
Z9	O34:O145:H45	rectal	1c					B1
Z16	O37:H10	rectal	1a–1c					B1
B1	O6:H16	rectal	1c					B1
Z20	O70:H10	rectal	1a–1c					B1
Z17	O76:H19	rectal	1a–1c					B1
Z21	O76:H19	rectal	1c					B1
Z26	O76:H19	rectal	1c					B1
Z32	O76:H19	rectal	1a–1c					B1
Z34	O76:H19	rectal	1a–1c	2b–2g				B1
E16	O8:NM	rectal	1c					B1
E18	O8:H2	rectal	1c–1d					B1
C28	O84:H14	rectal	1c					B1
C27	O91:H10	rectal	1c					B1
Z12	O91:H28	rectal	1a–1c					B1
Z13	O91:H42	rectal	1a–1c					B1
Z14	O91:H47	rectal	1a–1c					B1
Z18	O91:H47	rectal	1c					B1
C3	O96:H20	carcass	1c					B1
V5	O176:H21	rectal	1c	2g				B1
B3	O176:NM	rectal	1c					B2
B15	O6:NM	rectal	1c					C
V11	O153:NM	rectal	1c	2g				F

Isolates: unmarked STEC; * EPEC; # ETEC; ° EIEC. PG: Phylogenetic group; rectal: rectal swab; carcass: carcass swab; NM: no mobile; +: gene presence.

3.3. Virulence Genes and Pathotypes

Regarding the presence of STEC, 43/90 (47.7%) isolates had the *stx1* w/o *stx2* genes, therefore were assigned as STEC non-O157; only one isolate expressing *stx1* and *eae* genes was classified as t-STEC (typical STEC). Additionally, 3/90 harbored only the *eae* gene and were classified as EPEC, the *stp* gene was found in 2/90 isolates (2.2%) and were classified as ETEC, 1/90 (1.1%) isolates harbored the *ipaH,* and was classified as EIEC, and finally, the absence of a *aggr* gene revealed that no EAEC isolates were present.

3.4. Shiga Toxin Subtypes

The *stx1* and *stx2* genes and their subtypes were found in the STEC isolates as follows: in rectal swab (39/43, 90.6%) rather than carcass (4/43, 9.4%), and only one isolate from a rectal swab harbored *stx1-eae* (2.3%). Regarding *stx1* genes, *stx1c* only was found in 60.5% (26/43), followed by *stx1a-stx1c* 20.9% (9/43) and *stx1a-stx1d* 2.3% (1/43) (Table 4). The presence of both, *stx1* and *stx2* genes was found in 7/43 isolates (16.3%) from rectal swabs, and the combination *stx1c-stx2g* was detected in 3/43 isolates (6.9%), while 4 (9.4%) isolates showed different patterns (*stx1a-stx1c-stx2g*; *stx1c-stx2b-stx2g*; *stx1c-stx2b* and finally *stx1a-stx1c-stx2b-stx2g*) (Table 4).

3.5. Phylogroups

STEC isolates showed the major diversity of phylogenetic groups, although phylogroup B1 was predominant in 90.6% (39/43), while there was only one isolate (2.3%) in each remaining phylogroup (A, B2, C, and F). All EPEC, ETEC, and EIEC isolates were clustered in phylogroup B1 (Table 4). Phylogroups D and E were not found in the analyzed isolates.

3.6. Antimicrobial Resistance

The antimicrobial susceptibility profile of STEC, EPEC, ETEC, and EIEC was similar; all isolates expressed an antimicrobial resistance of 100% to NIT, followed by AMP (range of 66% to 100% according to pathotype), TET (30% to 100%), and the lowest for SXT (9% to 33%). Antimicrobial resistance was not observed in the other antibiotics. Almost all STEC, EPEC, ETEC, and EIEC showed multi-drug resistance (MDR) to at least three or four antibiotic classes used in this study (Table 5). We observed that 27.9% (12/43) of STEC isolates carried at least one antibiotic resistance: nine isolates expressed the *tetB* gene, one isolate the *tetA* gene, two isolates *the sul2* gene, one isolate the *sul1* and one isolate the *sul1-tetB* genes (Table 5).

Table 5. Antimicrobial resistance profile of diarrheagenic *E. coli* (DEC) isolated from sheep slaughtered in an abattoir.

Isolate	Serotype	Source	STEC	EPEC	ETEC	EIEC	Antimicrobial Resistance Profile	Resistance Gene			
								tetA	*tetB*	*sul1*	*sul2*
Z3	O146:H21	rectal	+				NIT, AMP, TET, SXT				+
V15	O176:NM	rectal	+				NIT, AMP, TET, SXT				
C28	O84:H14	rectal	+				NIT, AMP, TET, SXT				
V22	O100:H28	rectal	+				NIT, AMP, TET				
B7	O146:H8	rectal	+				NIT, AMP, TET		+		
V11	O153:NM	rectal	+				NIT, AMP, TET				
D26	O34:H14	carcass	+				NIT, AMP, TET		+		
E44	O53:H51	rectal	+				NIT, AMP, TET				
Z26	O76:H19	rectal	+				NIT, AMP, TET				
E16	O8:NM	rectal	+				NIT, AMP, TET				
V25	O100:H21	rectal	+				NIT, AMP				
D28	O104:H2	carcass	+				NIT, AMP		+		

Table 5. *Cont.*

Isolate	Serotype	Source	STEC	EPEC	ETEC	EIEC	Antimicrobial Resistance Profile	Resistance Gene			
								tetA	*tetB*	*sul1*	*sul2*
C23	O146:H21	rectal	+				NIT, AMP				
Z5	O174:H16	rectal	+				NIT, AMP				
Z25	O174:H16	rectal	+				NIT, AMP				+
B3	O176:NM	rectal	+				NIT, AMP				
C24	O185:NM	rectal	+				NIT, AMP		+	+	
E15B	O32:H27	rectal	+				NIT, AMP				
E20	O32:H7	rectal	+				NIT, AMP				
E30	O32:H7	rectal	+				NIT, AMP				
Z9	O34:O145:H45	rectal	+				NIT, AMP				
Z16	O37:H10	rectal	+				NIT, AMP				
B1	O6:H16	rectal	+				NIT, AMP				
B15	O6:NM	rectal	+				NIT, AMP				
Z17	O76:H19	rectal	+				NIT, AMP				
Z32	O76:H19	rectal	+				NIT, AMP		+		
Z34	O76:H19	rectal	+				NIT, AMP				
E18	O8:H2	rectal	+				NIT, AMP		+		
C27	O91:H10	rectal	+				NIT, AMP	+			
Z12	O91:H28	rectal	+				NIT, AMP				
Z13	O91:H42	rectal	+				NIT, AMP				
Z14	O91:H47	rectal	+				NIT, AMP				
Z18	O91:H47	rectal	+				NIT, AMP				
C3	O96:H20	carcass	+				NIT, AMP				
V13	O146:H10	rectal	+				NIT, TET				
V5	O176:H21	rectal	+				NIT, TET		+		
C28B	-:H14	rectal	+				NIT				
Z29	-:H16	rectal	+				NIT				
Z19	O146:H21	rectal	+				NIT				
B13C	O150:NM	carcass	+				NIT				
Z20	O70:H10	rectal	+				NIT				
Z21	O76:H19	rectal	+				NIT		+		
Z2	-:H34	rectal	+				NIT, AMP, TET, SXT		+		
D3	-:H34	carcass		+			NIT, AMP, TET, SXT				
Z15	-:H10	rectal		+			NIT, AMP				
D53	O28 AC:H21	carcass		+			NIT, AMP				
D15	O105 AB:H16	carcass			+		NIT, AMP, TET				
D15B	O120:H16	carcass			+		NIT, AMP				
E3	O28 AC:H21	rectal				+	NIT, AMP, TET				

NM: no mobile; NIT: nitrofurantoin; AMP: ampicillin; TET: tetracycline; SXT: trimethoprim-sulfamethoxazole; rectal: rectal swab; carcass: carcass swab.

4. Discussion

Different *E. coli* pathotypes are related to diarrhea in both human and animal populations with some serotypes capable of causing outbreaks [6,32]. In this work, several serotypes associated with diarrhea in humans in Mexico and other countries were found (O8:NM, O76:H19 and O146:H21) [32,33]. Moreover, serotypes O6:NM, O91:H10, and O104:H2 have been related to HUS. It is important to highlight that serotype O146:H21 was found in sheep from farms and slaughterhouses in Brazil [34,35], while the same serotype can be found in Mexico backyard sheep or adult sheep from Norway [36,37]. Similarly, the serogroup O104 is considered of clinical importance in the European Economic Community; interestingly, this serogroup is disseminated in lambs and sheep from India and lambs in Mexico [38–41].

According to Monaghan et al. (2011) [42], 40% of putative pathogenic *E. coli* belongs to pathotype STEC (non-O157) and is the major agent of microbial contaminations in meat products in Europe and USA [43,44]. In this work, STEC was the most frequent pathotype (47.7%) in sheep slaughtered in the abattoir; this finding is similar to that reported in Brazil

in sheep abattoirs (11.3%), or slaughter-age sheep from Australia (72%), and with other ruminants like goats in Kenya (50%), Iran (16.4%) or bovines in Burkina Faso (37%) and Mexico (40.7%) [14,16,34,45–47].

The most frequent *stx* subtype gene described worldwide in sheep is *stx1c* [48–50]. The results of our investigation corroborate this statement, however, a small number of isolates carried *stx1a* and *stx1d*. The *stx1c* subtype gene is related to diarrhea without complications in humans [9].

Recent research has shown that *stx* subtypes have a predilection toward different receptors: the Stx B subunit recognizes Gb3 as its principal receptor and to a lesser extent Gb4. Lee and Tesh 2019 [51] highlighted the relevance of this interaction as a key mechanism in the pathogenicity of STEC. Stx1a interacts strongly with Gb3 on the human glomerular endothelium. On the other hand, the subtype *stx2e* shows predilection for Gb4 and Gb5 present in the glomerular endothelium of ruminants and pigs.

In this work, the *stx2g* gene was predominant, followed by *stx2b*. These subtypes are not associated with HUS and HC development in humans, which could represent a low hazard to establish disease. In contrast, the presence of *stx2c* and *stx2d* genes that were not reported in this investigation that boost the development of HUS and HC, were reported in sheep carcasses from Turkey and Switzerland. Amezquita et al. (2014) [36] found *stx2c* and *stx2d* genes in backyard sheep in Mexico. Prager et al. (2011) [8] demonstrated that isolates harboring *stx2g* gene obtained from humans, animals and environmental sources had a close phylogenetic relationship, reinforcing the idea of human infections as a potential zoonotic disease.

Identification of *stx* subtypes is a priority, as it allows for an early prediction of the virulence potential of each STEC isolate. This observation generated enough evidence to know that *stx2a* and *stx2d* genes are crucial determinants in the severity of HUS; furthermore, the mere presence of the *stx2a* gene is considered an independent risk factor to the developed HUS in multivariate analysis. Therefore, the identification of *stx* subtypes should be performed routinely in diagnostic laboratories [52].

Pathotype EPEC was the second most frequent (3.3%) and is responsible for neonatal diarrhea in human and animal populations [53], however it also affects adult sheep in Australia and Brazil [34,45]. The isolates in this study did not express the *bfp* gene, so they were categorized as a-EPEC [6].

ETEC was the third most frequent pathotype (2.2%) and is considered one of the main diarrheagenic pathogens in lambs and calves [54]. In Kenya, it was also reported as the third most frequent DEC (10%) in slaughtered goats, while an investigation in Mexico rated it as the second most frequent in bovines, the same as in Burkina Faso (4%) [14,16,46]. Previous reports about ETEC were described in bovines with/without diarrhea in Brazil, Vietnam (with the same number of isolates as this work), and Burkina Faso [55–57]. There is a lack of information regarding this pathotype in slaughtered sheep in Mexico.

The last reported pathotype was EIEC with only 1.1%. This low percentage is also observed by other authors in comparison with other pathotypes in other species; for example, Navarro et al. (2018) [16] only discovered 11.5% of EIEC in bovine feces, while Kagambéga et al. (2012) [14] found 1% of this pathotype in slaughtered poultry in Burkina Faso.

In this work EAEC was not detected, however other investigations report this pathotype along with STEC, EPEC, and ETEC in Mexico, Iran, and Burkina Faso in bovines and goats [14,16,47].

The presence of most of the DEC pathotypes of public health concern can be isolated from sheep, goats, and bovines, which raises the relevance of livestock as a reservoir of these pathogens [58]; precarious hygiene conditions make it possible for DEC to contaminate meat products with feces during different processes in slaughterhouses in Mexico.

The high percentage (~90–100%) of isolates belonged to phylogenetic group B1 (commensal *E. coli*), which is similar to that reported in other countries in isolates from sheep, goats, and bovines [59–62].

Antimicrobial resistance (AMR) was observed in STEC isolates against AMP (72%), TET (30%), and SXT (9%); these percentages were lower in comparison to a study carried out in Turkey where a higher frequency of AMR to AMP and TET (100% and 50% respectively) was reported [63]. In Egypt, lower levels of AMR to AMP (66.7%) were reported, but higher levels of SXT (73.3%) were discovered in a goat slaughterhouse [64]. In Mexico, a study detected 92% and 75% of AMR to AMP and TET, respectively, in bovines. This contrasts with our study where both antibiotics showed a lower level of AMR. Another study in this country found AMR to cephalosporins in STEC isolates from bovines. Interestingly, we did not find any AMR to these antibiotics. Despite this, both studies showed AMR to TET and AMP [16,65].

In the case of a-EPEC, a lower resistance rate in comparison with this study was found in adult sheep in Spain with a 1.9%, 0, and 1% for GEN, TET, and SXT, respectively [66]. Conversely, a study from Brazil detected higher rates of resistance against CIP (22%), AMK (4%), GEN (9%) and cephalosporins (72%) in a sheep abattoir [67]. In the particular case of ETEC, we found higher resistance levels for AMP (100%) and TET (50%) in our work compared to Njoroge et al. (2013) [46] with goat isolates in Kenya.

Multi-drug resistance (MDR) was found against three or four antibiotic classes in 11 STEC, 1 EPEC, 1 ETEC and 1 EIEC isolates. The presence of MDR *E. coli* in the gut microbiota of the analyzed sheep could further disseminate to other microorganisms due to horizontal gene transfer [68].

In the present study, it was possible to detect resistance genes such as *tetA*, *tetB*, *sul1*, *sul2* within isolates resistant to tetracycline and trimethoprim-sulfamethoxazole. Research studies around the world also reported finding some of these genes. Portugal [69] informed the presence of *tetA*, *tetB* and, in a smaller number, *sul2*, in sheep samples processed in a slaughterhouse. Medina et al. (2011) [66], working with live sheep in Spain, reported the presence of these same genes with *tetA* the most frequent. Finally, in France, bovine isolates harbored *tetA and sul2* genes [15].

5. Conclusions

We identified several serotypes related to gastrointestinal illness in Mexico, along with some *stx* subtypes genes that were reported worldwide as low virulent (*stx1a*, *stx1c*, *stx1d*, *stx2b*, and *stx2g*). Nevertheless, some serotypes are implicated in diarrhea and MDR isolates could pose a threat for treatment in case of intestinal and extra-intestinal illness in people who consume sheep meat. These findings reflect the potential concern of sheep as a primary reservoir of STEC non-O157 and the possible transmission through the food chain.

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