ORIGINAL ARTICLE

Veterinary Research Forum. 2022; 13 (2) 169 – 176
doi: 10.30466/vrf.2020.128661.2977
Journal Homepage: vrf.iranjournals.ir

Veterinary Research Forum

Shiga toxin-producing *Escherichia coli* O157 in piglets and food from backyard systems

Gerardo Uriel Bautista-Trujillo[1]*, Mayra Isabel Hernández-Hernández[2], Javier Gutiérrez-Jiménez[3], Fernando Azpiri-Álvarez[1], Rene Pinto-Ruiz[2], Francisco Guevara-Hernández[2], Benigno Ruiz-Sesma[1], Paula Mendoza-Nazar[1], Daniel González-Mendoza[4]

[1] Department of Microbiology, Faculty of Veterinary Medicine and Zootechnics, Autonomous University of Chiapas, Chiapas, Mexico; [2] Faculty of Agronomic Sciences, Autonomous University of Chiapas, Chiapas, Mexico; [3] Institute of Biological Sciences, University of Sciences and Arts of Chiapas, Chiapas, Mexico; [4] Institute of Agricultural Sciences, Autonomous University of Baja California, Baja California, Mexico.

Article Info

Article history:

Received: 07 June 2020
Accepted: 03 November 2020
Available online: 15 June 2022

Keywords:

Antimicrobial susceptibility
Carrier
Diarreagenic *Escherichia coli*
Prevalence
Zoonosis

Abstract

Piglets suffer from diarrhea caused by the Shiga toxin-producing *Escherichia coli* (STEC) and can be carriers of the bacteria, with public health consequences in developing countries. The aim of the present study was to study the prevalence of STEC O157 in feces of 465 piglets and 54 food mixes from backyard systems, the antimicrobial susceptibility of STEC and the frequency of genes encoding extended-spectrum β-lactamases. The *E. coli* was isolated from 75.90 % of the evaluated feces. The STEC strains were identified in 33.11% of the sampled population and in 43.60% of the piglets carrying *E. coli*. Among STEC strains, the *stx*1 gene was the most frequent (22.30%). The *rfb*O157 gene was amplified in 47.40% of the STEC strains. High frequencies of STEC strains were not susceptible to ampicillin, carbenicillin and tetracycline. The *bla*TEM gene (52) was the most frequent among strains not susceptible to ampicillin. Class 1 integrons were the most frequent in those strains. Of the identified STEC strains, 48.70% were considered as multi-drug resistant and 1.90% were considered extensively drug resistant. In the supplied food, STEC O157 strains were identified in 25.00% of the STEC strains. We conclude that the piglets from backyard systems are carriers of STEC O157 strains not susceptible to common antibiotics, including penicillins and tetracyclines. In addition, supplied food is a source of this type of pathogenic bacteria. Through their direct contact with humans, the piglets and food represent a potential source of bacterial dissemination capable of producing gastrointestinal infections in humans.

Introduction

Escherichia coli is a zoonotic agent with the greatest impact on swine systems, causing acute enteritis with watery diarrhea in post-weaning piglets.[1] The high mortality and medication of piglets cause economic losses ranging from 25.00 to 50.00% of the profits of swine systems. [2] The pathogenic strains of *E. coli* in pigs include Shiga toxin-producing *E. coli* (STEC).[3] The serotype O157:H7 of STEC has become an important pathogen causing diarrhea, hemorrhagic colitis and hemolytic uremic syndrome in humans throughout the world.[4] Currently, the main concern of researchers is the increasing spread of *E. coli* strains carrying a group of β-lactamases known as extended-spectrum β-lactamases (ESBLs) having the ability to cause resistance to new beta-lactam antibiotics and other antibiotic families.[5,6]

Some studies have examined the prevalence of O157 and non-O157 STEC strains in piglets in Mexico.[7,8] However, most of these studies were carried out with piglets of intensive and semi-intensive swine farms, in which there is a strict control of sanitary measures and not much has been studied in swine backyard systems.

Swine backyard farming is a production system characteristic of certain regions of Mexico and other countries in the world representing a source of income

***Correspondence:**

Gerardo Uriel Bautista Trujillo. PhD
Department of Microbiology, Faculty of Veterinary Medicine and Zootechnics, Autonomous University of Chiapas, Chiapas, Mexico
E-mail: gerardo.trujillo@unach.mx

and animal protein for families in rural communities. Despite its benefits, this type of systems is associated with a high risk of contamination by pathogens and an inappropriate use of antibiotics.[9]

Whether the piglets and food supplied in backyard systems can carry STEC O157 with antibiotic resistance mediated by ESBL has not been previously investigated and it was the main motivation for this study. The goal of this study was to investigate the prevalence of STEC O157 in piglets' feces and food supplied in backyard systems in Chiapas, Mexico, the antimicrobial susceptibility of STEC strains and the frequency of genes encoding ESBL.

Materials and Methods

Study population. The study population consisted of 465 healthy hybrid (Yorkshire x Duroc) piglets (from 1 to 6 weeks of age) being randomly selected. The study period lasted from the winter of 2016 to the summer of 2018. Animal studies were approved by the Ethics Committee of the University of Sciences and Arts of Chiapas (approval #049/02-2018). All piglets came from backyard farms being located in Chiapas, Mexico. The average number of pigs in each pen was between one and ten. These piglets were maintained in pens made of wooden or masonry walls having roofs made from metal sheets or locally found materials, dirt or concrete floors, simple drinkers and feeders made from hollowed-out trunks, with no waste treatment system in place. Agricultural residues and food waste from homes, restaurants and markets constitute the food source. Fifty-four samples (150 g each) of a food mix (tortillas, fruits and vegetables) were collected, placed in sterilized plastic bottles and transported to the laboratory.

Isolation of *E. coli.* The samples were taken directly from the rectum of piglets using sterile swabs and placed in Stuart's medium (Copan Diagnostics, Murrieta, USA); the swabs and bottles were transported in cold chain for microbiological analysis. Sub-samples (100 g) from each sample of food mix were placed in a sterile plastic bag and lactose broth was added to reach a 1:10 (10^{-1}) final dilution.[10] These sub-samples were mixed for 1 min. The swabs and foods dilution were simultaneously inoculated onto the eosin-methylene blue agar (Sigma-Aldrich, Steinheim, Germany) and incubated at 37.00 ˚C for 24 hr. The lactose-fermenting colonies were subjected to the series of biochemical tests to confirm the identity of *E. coli* strains. The identity of *E. coli* strains was also genetically confirmed by polymerase chain reaction (PCR) through the amplification of *uid*A gene.[11] MacConkey agar with sorbitol (Sigma-Aldrich) was used to detect the serotype O157:H7 of STEC.[12]

Identification of STEC by PCR. In the PCR, the *E. coli* strain ATCC® 25922™ was used as a negative control and the STEC EDL933 strain (O157:H7) as a positive control. The strains were provided by Dr. Teresa Estrada García of CINVESTAV, Mexico, and deposited in the bacterial collection of University of Sciences and Arts of Chiapas, Chiapas, Mexico.[13] To obtain DNA, bacterial lysates from each of the previously selected colonies were prepared, suspended in 1.00 mL of deionized water and then boiled for 10 min. The bacterial lysates were centrifuged at 10,000 rpm for 5 min; the supernatant containing DNA was removed and stored at – 80.00 ˚C. The gene primers specific for Shiga toxin-producing *E. coli* (*stx*1 and *stx*2 genes) were amplified by PCR.[14] Shiga toxin-producing *E. coli* O157 was studied by amplifying the *rfb* gene (specific O-polysaccharide).[15] The amplification of ESBL genes *bla*TEM, *bla*SHV, *bla*CTXM, *bla*OXA and *bla*CMY was carried out using the primers and conditions reported previously.[16] The primers for amplification of class 1 and 2 integrons genes were used as described by Mazel et *al*., and White *et al*., respectively.[17,18] The primer sequences used in this study are provided in Table 1. The PCR reactions were run in a thermal cycler C1000 (Bio-Rad Laboratories, Hercules, USA) and the PCR products were analyzed through agarose gel electrophoresis (2.00%) at 80.00 V for 1 hr. The agarose gels were stained with Sybr Green® (Invitrogen, Carlsbad, USA) and visualized with the Molecular Imager® Gel Doc™ XR System (Bio-Rad). Lambda molecular weight markers (10 and 1000 bp; Invitrogen) were also used.

Antimicrobial susceptibility analysis. The disk diffusion method was performed following the recommendations of Clinical and Laboratory Standards Institute.[19] The following antimicrobial susceptibility discs (BD BBL™ Sensi-Disc™, Becton, Dickinson and Company, San Jose, USA) were used for different anti-microbial categories: β-lactamic: ampicillin (10.00 µg), carbenicillin (100 µg) and oxacillin (1.00 µg), aminoglycosides: amikacin (30.00 µg), netilmicin (30.00 µg) and gentamicin (10.00 µg), cephalosporins: cefalotin (30.00 µg) and cefotaxime (30.00 µg), quinolones: ciprofloxacin (5.00 µg) and norfloxacin (10.00 µg), phenicols: chloramphenicol (30.00 µg), folate inhibitors: trimethoprim-sulfamethoxazole (25.00 µg), furans: nitrofurantoin (300 µg) and tetracyclines: tetracycline (30.00 µg). The β-lactam-resistant strains were subsequently analyzed using the disc diffusion method with amoxicillin-clavulanic acid discs (20.00/10.00 µg). The *E. coli* strains (intermediate and resistant phenotypes) not susceptible to at least three antibiotics belonging to different antimicrobial categories were classified as multi-drug resistant strains (MDRs); while, the strains not susceptible to at least one antibiotic belonging to each of the tested antimicrobial categories were classified as extensively drug resistant (XDR).[20]

Table 1. Primers used in this study.

Primer pair	Sequence (5'-3')	Encoded protein	Size (pb)	Reference
uidA	F: AAAACGGCAAGAAAAAGCAG R: ACGCGTGGTTAACAGTCTTGCG	β-glucuronidase	147	11
Stx1	F: CTGGATTTAATGTCGCATAGTG R: AGAACGCCCACTGAGATCATC	Shiga toxin 1	150	14
Stx2	F: GGCACTGTCTGAAACTGCTCC R: TCGCCAGTTATCTGACATTCTG	Shiga toxin 2	255	14
***rfb*O157**	F: CGGACATCCATGTGATATGG TTGCCTATGTACAGCTAATCC	Specific O-polysaccharide	259	15
blaTEM	F: ATAAAATTCTTGAAGACGAAA R: GACAGTTACCAATGCTTAATC	beta-lactamase TEM	1080	16
blaSHV	F: TTATCTCCCTGTTAGCCACC R: GATTTGCTGATTTCGCTCGG	beta-lactamase SHV	795	16
blaCTXM	F: CGCTTTGCGATGTGCAG R: ACCGCGATATCGTTGGT	beta-lactamase SHV	550	16
blaOXA	F: TCAACTTTCAAGATCGCA R: GTGTGTTTAGAATGGTGA	beta-lactamase OXA	591	16
blaCMY	F: GACAGCCTCTTTCTCCACA R: TGG AACGAAGGCTACGTA	beta-lactamase CMY	1000	16
IntI	F: GGGTCAAGGATCTGGATTTCG R: ACATGCGTGTAAATCATCGTCG	*int*I1	483	17
Int2	F: CGGGATCCCGGACGGCATGCACGATTTGTA R: GATGCCATCGCAAGTACGAG	class 2 integron	variable	18

Results

A total of 353 (75.90%) strains of *E. coli* were isolated from fecal samples collected from 465 piglets. The STEC strains were identified in 33.11% (154/465) of the sampled piglets and detected in 43.60% (154/353) of the piglets carrying *E. coli*. Among STEC strains, 22.30% (79/154) strains were carriers of the *stx*1 gene, 6.80% (24/154) of the *stx*2 gene and 14.40% (51/154) of both the *stx*1 and the *stx*2 genes. The *rfb*O157 genetic marker was amplified by PCR in 47.40% (73/154) of the STEC strains. In the supplied food, STEC strains were isolated from 14.80% (8/54) of the sampled food mix and identified in 16.00% (8/50) of the sampled food mix carrying *E. coli*; while, the *rfb*O157 genetic marker was amplified in 25.00% (2/8) of the STEC strains.

Antimicrobial susceptibility profile. Susceptibility of STEC strains to antibiotics used to treat gastrointestinal infections caused by *E. coli* was evaluated. More than three thirds of the identified STEC strains were not susceptible to ampicillin and carbenicillin. Half of the strains were not susceptible to tetracycline. The susceptibility of O157 strain was also evaluated; more than three thirds of the strains were not susceptible to ampicillin, carbenicillin and tetracycline (Table 2). The frequency of genes encoding β-lactamase in all 142 STEC strains not susceptible to ampicillin was also assessed. The *bla*TEM gene (52) was the most frequent among STEC strains, followed by *bla*CTX (25) and *blaSHV* (8). Seventeen STEC strains not susceptible to ampicillin turned out to be carriers of both the *bla*TEM genes and *bla*CTX genes; while, five strains were carriers of the *bla*TEM, *bla*CTX and *bla*SHV genes (Table 3).

Table 2. Antimicrobial non-susceptibility profile of the Shiga toxin-producing *Escherichia coli* (STEC) strains.

Antimicrobial	Percentage of non-susceptibility (n)				
	STEC (n = 154)	*stx*1 (n = 79)	*stx*2 (n = 24)	*stx*1/*stx*2 (n = 51)	O157 (n = 73)
Ampicillin	81.10 (125)	89.80 (71)	83.30 (20)	66.60 (34)	91.70 (67)
Amoxicillin-clavulanic acid	25.30 (39)	26.50 (21)	20.80 (5)	25.50 (13)	43.80 (32)
Carbenicillin	66.80 (103)	67.10 (53)	70.80 (17)	64.70 (33)	82.10 (60)
Oxacillin	5.80 (9)	3.80 (3)	8.30 (2)	7.80 (4)	10.90 (8)
Amikacin	20.70 (32)	17.70 (14)	25.00 (6)	23.50 (12)	36.90 (27)
Gentamicin	15.60 (24)	12.60 (10)	20.80 (5)	17.60 (9)	28.70 (21)
Netilmicin	11.70 (18)	11.40 (9)	16.70 (4)	9.80 (5)	19.10 (14)
Cefalotin	26.60 (41)	25.30 (20)	33.30 (8)	25.50 (13)	42.40 (31)
Cefotaxime	11.00 (17)	11.40 (9)	12.50 (3)	9.80 (5)	23.20 (17)
Ciprofloxacin	11.70 (18)	11.40 (9)	16.70 (4)	9.80 (5)	21.90 (16)
Norfloxacin	14.90 (24)	16.40 (13)	25.00 (6)	9.80 (5)	30.40 (22)
Chloramphenicol	27.90 (43)	32.90 (26)	20.80 (5)	23.50 (12)	34.20 (25)
Trimethoprim-sulfamethoxazole	21.40 (33)	25.30 (20)	29.00 (7)	11.70 (6)	35.60 (26)
Nitrofurantoin	8.40 (12)	10.30 (8)	8.30 (2)	3.90 (2)	12.30 (9)
Tetracycline	48.00 (74)	43.00 (34)	62.50 (15)	49.00 (25)	83.50 (61)

Table 3. Genes of extended spectrum β-lactamase producing Shiga toxin-producing *Escherichia coli* (STEC) strains isolated from piglets.

STEC groups (n)	Non-susceptible profile β-lactamic: Ampicillin	β-lactamase gene	Number of isolates
***STEC stx1* (79)**	71	*TEM*	32
		CTX	13
		SHV	5
		TEM+CTX	8
		TEM+CTX+SHV	2
***STEC stx2* (24)**	20	*TEM*	5
		CTX	4
		SHV	1
		TEM+CTX	3
		TEM+CTX+SHV	2
***STEC stx1/stx2* (51)**	51	*TEM*	15
		CTX	8
		SHV	2
		TEM+CTX	6
		TEM+CTX+SHV	1

Of the identified STEC strains, 48.70% (n = 75) were not susceptible to at least one antibiotic in three different antimicrobial categories; these strains were considered as MDR. Also, 1.90% (n = 3) of STEC strains, predominantly STEC stx2, were not susceptible to at least one antibiotic in all tested categories; these strains were considered XDR. Class 1 integrons were detected in 74 STEC strains from 142 isolates not susceptible to ampicillin. Class 2 integrons were not detected (Table 4). The susceptibility of STEC strains isolated in food mix was evaluated. All the identified STEC strains were not susceptible to ampicillin and carbenicillin. Eight STEC strains not susceptible to ampicillin turned out to be carriers of the *bla*TEM gene; while, two STEC strains not susceptible to ampicillin turned out to be carriers of both the *bla*TEM genes and *bla*CTX genes. Of the identified STEC strains (n = 8), two strains were considered as MDR, class 1 integrons were detected in four STEC strains and class 2 integrons were not detected (data not showed).

Discussion

Swine backyard farming systems are common in Mexico and developing countries worldwide. However, important problems have been described in these production systems, such as the lack of adequate technologies and technical assistance, which leads to a high prevalence of diseases.[21] This is the first study conducted in Mexico that reports the presence of STEC (43.60%) carrying the *stx*1 and/or *stx*2 genes in piglets from backyard systems. Of these STEC strains, 47.40% amplified the *rfb*O157 genetic marker. In contrast, another study has shown low presence of the *stx*1 and *stx*2 genes (0.10% and 1.00%, respectively) in STEC strains isolated from piglets of farms located in the central region of Mexico.[7] In this context, a low prevalence (2.10%) of *E. coli* O157 was reported in pigs from farms located in the central region of Mexico.[8] Unlike intensive and semi-intensive swine systems, in which there is a strict control of the personnel and application of sanitary measures, backyard systems are characterized by poor animal health management and, in many cases, no biosecurity measures, explaining the contrast in these results.

Our hypothesis is that the acquisition and dissemination of STEC O157 and non-O157 strains in piglets from backyard systems could be related to the origin of the food provided to pigs and to direct contact between pigs, humans and pets. Unlike specialized farms, in backyard systems the pigs diet is based on fruit and vegetable waste, stale tortillas and bread, etc. This variety of ingredients is associated with a greater variability of the intestinal bacterial population, which is considered beneficial to the health of host.[22] However, there is a high risk of contamination with the pathogens present in the pigs' food due to poor sanitary management. Pathotypes of diarrheagenic *E. coli*, including STEC, have been identified in ready-to-eat cooked vegetable salads (1.40%) distributed by restaurants in Mexico[23] and tomatoes (6.00%) purchased from public markets in Pachuca, Mexico.[24] These results are consistent with the findings of our work, suggesting that STEC could be acquired and disseminated through the vegetable waste provided as a feed to pigs. Although the sample size analyzed here was small, we did detect STEC O157 in supplied food, indicating that these foods represent a potential source of bacterial dissemination for piglets. Swine backyard farming systems are characterized by the involvement of women and other family members in animal management activities as well as people outside the family during the sale process. Direct contact between humans and animals is a major factor in the spread of STEC, especially in developing countries with a high prevalence of gastrointestinal infections in humans caused by diarrheagenic *E. coli*.[25,26] Moreover, the presence of pets and other domestic animals (cattle, sheep and birds) inside the house as well as proliferation of harmful fauna are also factors involving in the spread of harmful germs, since this type of animals are important reservoirs of diarrheagenic *E. coli*, including the O157:H7 serotype, participating in gastrointestinal infections in humans.[27,28]

Table 4. Non susceptible profiles in STEC strains isolates from piglets.

Class (No.)	Non susceptible phenotype	No.	ESBL gene (No.)	Integron class	Genetic marker
STEC stx1 (n=79)					
(0)	0	4			
(1)	AMP	9	TEM (4), CTX (5)		
(1)	CAR	1			
(1)	AMP CAR	13	TEM (7), CTX (6)		
(2)	AMP STX	1		Class 1	
(2)	AMP CEF	1			
(2)	AMP CHL	1			
(2)	AMP NIT	1			
(2)	AMP CAR CEF	2	TEM	Class 1	
(2)	AMP AMK CAR	2	TEM	Class 1	
MDR (3)	AMP STX TET	1	CTX	Class 1	O157
(2)	AMP CAR TET	1	TEM	Class 1	
MDR (3)	CHL STX TET	1		Class 1	O157
(2)	AMP CAR CHL	3	TEM (3)		
MDR (3)	AMP CHL TET	1	TEM	Class 1	
MDR (3)	AMP CAR CHL TET	1	TEM	Class 1	O157
MDR (3)	CAR CEF CTX NOR	1			O157
MDR (3)	AMP AMC CHL TET	1	TEM	Class 1	O157
MDR (3)	AMP CAR STX TET	1	TEM	Class 1	O157
MDR (3)	AMP CAR CHL TET	2	SHV (1)	Class 1	
MDR (4)	CAR CEF CHL TET	1		Class 1	O157
(2)	AMP AMC CAR CEF	1	TEM		O157
MDR (3)	AMP AMK CEF CTX	1	TEM		O157
MDR (4)	AMP CHL STX TET	1	CTX	Class 1	O157
MDR (3)	AMP CAR CTX TET	1	TEM	Class 1	O157
MDR (3)	AMP AMC CAR STX TET	1	TEM	Class 1	O157
MDR (3)	AMP AMC AMK CAR TET	1	CTX	Class 1	O157
MDR (3)	AMP CAR CEF CTX NET	1	SHV		O157
MDR (4)	AMP AMK CEF STX TET	1	TEM	Class 1	O157
MDR (5)	AMP CAR NOR CHL STX TET	1	TEM	Class 1	O157
MDR (3)	AMP AMC CAR CIP NOR TET	1	CTX	Class 1	O157
MDR (5)	AMP AMC AMK CEF STX TET	1	SHV	Class 1	O157
MDR (4)	AMP AMC CAR CEF CHL STX	1	TEM	Class 1	
MDR (4)	AMP AMK CAR GEN NET CTX TET	1	TEM	Class 1	O157
MDR (5)	AMP AMC CAR AMK GEN CIP NOR	1	CTX		O157
MDR (5)	AMP AMC CAR AMK GEN CEF NET	1	TEM	Class 1	O157
MDR (6)	AMP AMC CAR CHL STX NIT TET	3	TEM+CTX+ SHV	Class 1	
MDR (5)	AMP AMC CAR OXA CEF CHL STX TET	1	TEM+CTX	Class 1	
MDR (6)	AMP NET CTX CIP NOR CHL TET	1	TEM	Class 1	O157
MDR (6)	AMP AMC CAR AMK GEN CEF CHL TET	1	CTX	Class 1	O157
MDR (6)	AMP AMC CAR CEF CHL STX NIT TET	1	TEM	Class 1	O157
MDR (7)	AMP CAR GEN CEF CIP NOR STX NIT TET	1	TEM+CTX	Class 1	O157
MDR (5)	AMP CAR GEN NET CEF CTX CIP NOR TET	1	TEM+CTX	Class 1	O157
MDR (6)	AMP AMC CAR AMK CIP NOR CHL NIT TET	1	TEM+CTX	Class 1	O157
MDR (5)	AMP AMC CAR AMK GEN NET CEF NOR TET	1	TEM+CTX+SHV	Class 1	O157
MDR (7)	AMP AMC CAR AMK NET NOR CHL STX NIT TET	1	TEM+CTX	Class 1	O157
MDR (7)	AMP AMC CAR OXA GEN NET CIP NOR CHL STX TET	1	TEM+CTX	Class 1	O157
MDR (7)	AMP AMC CAR OXA AMK GEN CEF CTX CIP NOR CHL STX TET	1	TEM+CTX	Class 1	O157
MDR (7)	AMP AMC CAR GEN NET CEF CTX CIP NOR CHL STX TET	1	TEM+CTX+SHV	Class 1	O157
STEC stx2 (24)					
(0)	0	(2)			
(1)	AMP	(4)			
(1)	AMP CAR	(1)			
MDR (3)	CAR CHL TET	(1)		Class 1	O157
(2)	AMP CAR TET	(2)	TEM	Class 1	O157
MDR (3)	CAR NOR CHL	(1)			O157
MDR (3)	AMP CAR NOR STX	(1)	TEM	Class 1	O157

Continued on next page

Table 4 Continued.

MDR (3)	AMP CAR NET TET	(1)	CTX	Class 1	O157
MDR (3)	AMP CAR CEF TET	(2)	TEM (1), CTX (1)	Class 1	
MDR (3)	AMP CAR SXT TET	(1)	SHV	Class 1	
MDR (4)	AMP CEF SXT TET	(1)	CTX	Class 1	O157
MDR (3)	AMP CAR CEF TET	(1)	TEM	Class 1	O157
MDR (4)	AMP AMC CAR AMK GEN TET	(1)	TEM+CTX	Class 1	O157
MDR (4)	AMP AMK CAR GEN STX TET	(1)	CTX	Class 1	O157
MDR (6)	AMP AMC CAR OXA AMK GEN NET CEF CTX CIP NOR CHL TET	(1)	TEM+CTX	Class 1	O157
MDR (6)	AMP AMC CAR AMK GEN NET CEF CTX CIP NOR SXT TET	(1)	TEM+CTX+SHV	Class 1	O157
XDR (8)	AMP AMC CAR AMK NET CEF CIP NOR CHL SXT NIT TET	(1)	TEM+CTX	Class 1	O157
XDR (8)	AMP AMC CAR OXA AMK GEN CEF CTX CIP NOR CHL SXT NIT TET	(1)	TEM+CTX+SHV	Class 1	O157
STEC stx1/stx2 (n=51)					
(0)	0	(10)			
(1)	AMP	(3)			
(1)	CHL	(2)			
(1)	CAR	(2)			
(1)	AMP CAR	(3)	TEM (3)		
(2)	CEF TET	(1)			
(1)	AMP AMK CAR	(1)	CTX		O157
(2)	AMP AMK GEN	(1)	CTX		
(2)	AMP CAR GEN	(1)	CTX		
(2)	AMP CAR TET	(1)	TEM	Class 1	O157
(2)	AMP CAR CHL	(1)	CTX		O157
(2)	AMP CAR GEN NET	(1)	CTX		O157
MDR (3)	AMP CAR CIP TET	(1)	SHV	Class 1	O157
MDR (3)	AMP CAR CEF TET	(1)	SHV	Class 1	O157
MDR (4)	CAR CHL STX TET	(1)		Class 1	O157
MDR (3)	AMP CAR CHL TET	(2)	TEM (2)	Class 1	O157
MDR (4)	AMP CAR AMK CEF TET	(1)	TEM	Class 1	O157
MDR (4)	AMP CAR CIP STX TET	(1)	TEM	Class 1	O157
MDR (4)	AMP CAR AMK GEN TET	(1)	CTX	Class 1	O157
MDR (3)	AMP AMC CAR OXA CIP TET	(1)	CTX	Class 1	O157
MDR (4)	AMP CAR CHL STX TET	(1)	TEM	Class 1	O157
MDR (3)	AMP CAR GEN NET CEF	(1)	TEM	Class 1	O157
MDR (4)	AMP CAR AMK CTX TET	(1)	CTX	Class 1	O157
MDR (3)	AMP AMC CAR AMK GEN TET	(1)	TEM	Class 1	
MDR (4)	AMP AMC AMK CEF CTX TET	(1)	TEM	Class 1	O157
MDR (4)	AMP AMC CAR CHL NIT TET	(1)	TEM+CTX	Class 1	O157
MDR (5)	AMP AMC CAR CEF NOR STX TET	(1)	TEM	Class 1	O157
MDR (4)	AMP AMC CAR OXA CIP NOR CHL TET	(1)	TEM+CTX	Class 1	O157
MDR (5)	AMP AMC CAR AMK CEF CHL TET	(1)	TEM+CTX	Class 1	O157
MDR (5)	AMP AMC CAR AMK CEF NOR TET	(1)	TEM	Class 1	O157
MDR (4)	AMC AMK CAR CEF CTX CHL TET	(1)	TEM	Class 1	O157
MDR (5)	AMP AMC CAR GEN NET CEF STX TET	(1)	TEM+CTX	Class 1	O157
MDR (5)	AMP AMC CAR AMK CEF CTX NOR TET	(1)	TEM+CTX	Class 1	O157
MDR (5)	AMP AMC CAR OXA AMK GEN NET CEF NOR TET	(1)	TEM+CTX	Class 1	O157
XDR (8)	AMP AMC CAR OXA GEN NET CEF CTX CIP CHL STX NIT TET	(1)	TEM+CTX+SHV	Class 1	O157

MDR; Multi Drug-Resistant, XDR; Extensively Drug-Resistant; AMP: Ampicillin, AMC: Amoxicillin, AMK: Amikacin, CAR: Carbenicillin, OXA: Oxacillin, GEN: Gentamicin, NET: Netilmicin, CEF: Cefalotin, NOR: Norfloxacin, CTX: Cefotaxime CIP: Ciprofloxacin, CHL: Chloramphenicol, STX: Trimethoprim-sulfamethoxazole, NIT: Nitrofurantoin TET: Tetracycline.

In this work, STEC strains were resistant mainly to ampicillin (81.10%), followed by carbenicillin (66.80%) and tetracycline (48.00%). This trend was similar for STEC O157. In addition, about half of the identified STEC strains were resistant to at least one antibiotic in three of the tested antimicrobial categories (MDR). The results of the present work showed a high frequency of resistance mainly to β-lactams in STEC strains isolated form piglets of backyard systems, similar to what being reported by other authors in intensive and semi-intensive swine systems. For example, high resistance to tetracycline (79.57%) and ampicillin (48.79%) was reported in STEC strains isolated from pig feces of farms located in the city of Chongqing, China.[29] Recently, the phenotype of resistance to ampicillin (99.50%) and carbenicillin (99.00%) was identified in STEC strains isolated from pigs of farms located in central

Thailand.[30] It has been reported that some beta-lactam antibiotics, such as penicillin and ampicillin, lose viability when use as a first-line of choice during chemotherapeutic treatment of an infectious process affecting pigs throughout the world due to the acquisition of resistance mechanisms.[5] The present study demonstrated the presence of genes encoding β-lactamase in STEC strains isolated from piglets of backyard systems, mainly the *bla*TEM (52), *bla*CTX (25) and *bla*SHV (8) genes. Moreover, class 1 integrons were also identified. These findings are consistent with those reported by Samanta *et al*.[31] The presence of *E. coli* with high resistance to ampicillin is common in piglets due to the presence of ESBLs *bla*CTX-M and *bla*TEM and class 1 integrons.[32,33] The results confirm that backyard piglets can be a carrier of ESBL- producing *E. coli*; however, further studies regarding the presence of specific *bla* profile are suggested. Bacteria carrying class 1 integrons play a role in the spread of resistance genes and pose a serious health risk to humans if transmitted to them.[34]

The present study showed that piglets from backyard systems are carriers of STEC O157 and non-O157 strains not susceptible to penicillins and tetracyclines. It also showed that the most of these strains have genes that code ESBLs, mainly *bla*TEM, *bla*CTX and *bla*SHV. In addition, we showed that STEC O157 and non-O157 could be acquired and disseminated through the food mix provided to pigs. These results could be used for the development of more efficient preventive measures, diagnostic methods and antimicrobial alternatives in swine backyard farming systems, in order to reduce a risk for public health.

Acknowledgment

Thanks to Edgar Ruiz Sánchez and Diana Laura Vázquez Vázquez for their collaboration in field and laboratory work. Thanks are also given to the people working in swine backyard farms in Chiapas, Mexico, for their collaboration with this study.

Conflicts of interest

The authors declare that the study was carried out in the absence of commercial or financial relationships that could be interpreted as a potential conflict of interest and all persons gave their informed consent prior to their inclusion in the study.

References

1. Heo JM, Opapeju FO, Pluske J R, et al. Gastrointestinal health and function in weaned pigs: a review of feeding strategies to control post-weaning diarrhoea without using in-feed antimicrobial compounds. J Anim Physiol Amim Nutr (Berl) 2013; 97(2): 207-237.
2. Malgarin CM, Takeuti KL, de Lara AC, et al. Virulence factors and antimicrobial resistance profile of *Escherichia coli* isolated from nursery piglets and drinking water. Acta Sci Vet 2018; 46(1): 6. doi:10.22456/1679-9216.81810.
3. Yang GY, Guo L, Su JH, et al. Frequency of diarrheagenic virulence genes and characteristics in *Escherichia coli* isolates from pigs with diarrhea in china. Micro-organisms 2019; 7(9): 308. doi: 10.3390/micro-organisms7090308.
4. Ibama O, Ibegbulem EO, Onwuli D, et al. The health implication of enterohaemorrhagic *Escherichia coli* (EHEC) 0157: H7: A review on haemolytic uraemic syndrome. AJRIMPS 2019; 7(4): 1-10.
5. Ungureanu V, Corcionivoschi N, Gundogdu O, et al. The emergence of beta-lactamase producing *Escherichia coli* and the problems in assessing their potential contribution to foodborne illness: a review. AgroLife Sci J 2019; 8(1): 248-260.
6. Smith MG, Jordan D, Gibson JS, et al. Phenotypic and genotypic profiling of antimicrobial resistance in enteric *Escherichia coli* communities isolated from finisher pigs in Australia. Aust Vet J 2016; 94(10): 371-376.
7. Toledo A, Gómez D, Cruz C, et al. Prevalence of virulence genes in *Escherichia coli* strains isolated from piglets in the suckling and weaning period in Mexico. J Med Microbiol 2012; 61(Pt 1): 148-156.
8. Callaway TR, Anderson RC, Tellez G, et al. Prevalence of *Escherichia coli* O157 in cattle and swine in central Mexico. J Food Prot 2004; 67(10): 2274-2276.
9. Montero-López EM, Martínez-Gamba RG, Herradora-Lozano MA, et al. Alternatives for small-scale pig production [Spanish]. Coyoacan, Mexico: National Autonomous University of Mexico. 2015; 17-35.
10. Cerna-Cortés JF, Gómez-Aldapa CA, Rangel-Vargas E, et al. Presence of indicator bacteria, *Salmonella* and diarrheagenic *E. coli* pathotypes on mung bean sprouts from public markets in Pachuca, Mexico. Food Control 2013; 31(2): 280-283.
11. Tsai YL, Palmer CJ, Sangermano LR. Detection of *Escherichia coli* in sewage and sludge by polymerase chain reaction. Appl Environ Microbiol 1993; 59(2): 353-357.
12. Tarr PI, Gordon CA, Chandler WL. Shiga-toxin-producing *Escherichia coli* and haemolytic uraemic syndrome. Lancet 2005; 365(9464): 1073-1086.
13. Gutiérrez-Jiménez J, Luna-Cazárez LM, Mendoza-Orozco MI, et al. Organization, maintenance, and preservation of the bacterial culture collection of the biological sciences institute, university of science and arts of Chiapas (UNICACH), Mexico [Spanish]. Rev Soc Ven Microbiol 2015; 35(2): 95-102.
14. López-Saucedo C, Cerna JF, Villegas-Sepulveda N, et al. Single multiplex polymerase chain reaction to detect

diverse loci associated with diarrheagenic *Escherichia coli*. Emerg Infect Dis 2003; 9(1): 127-131.
15. Paton AW, Paton JC. Detection and characterization of Shiga toxigenic *Escherichia coli* by using multiplex PCR assays for stx1, stx2, eaeA, enterohemorrhagic *E. coli* hlyA, rfbO111, and rfbO157. J Clin Microbiol 1998; 36(2): 598-602.
16. Ahmed AM, Motoi Y, Sato M, et al. Zoo animals as reservoirs of gram-negative bacteria harboring integrons and antimicrobial resistance genes. Appl Environ Microbiol 2007; 73(20): 6686-6690.
17. Mazel D, Dychinco B, Webb VA. Antibiotic resistance in the ECOR collection: integrons and identification of a novel aad gene. Antimicrob Agents Chemother 2000; 44(6): 1568-1574.
18. White PA, McIver CJ, Rawlinson WD. Integrons and gene cassettes in the enterobacteriaceae. Antimicrob Agents Chemother 2001; 45(9): 2658-2661.
19. Wayne PA. Performance standards for antimicrobial susceptibility testing; 24th informational supplement. Document M100-S24 Clinical and laboratory standards institute (CLSI) 2014: 50,110.
20. Magiorakos AP, Srinivasan A, Carey RB, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 2012; 18(3): 268-281.
21. Saavedra-Montañez M, Carrera-Aguirre V, Castillo-Juárez H, et al. Retrospective serological survey of influenza viruses in backyard pigs from Mexico City. Influenza Other Respir Viruses 2013; 7(5): 827-832.
22. Chen L, Wang L, Yassin AK, et al. Genetic characterization of extraintestinal *Escherichia coli* isolates from chicken, cow and swine. AMB Express 2018; 8(1): 117. doi:10.1186/s13568-018-0646-8.
23. Bautista-De León H, Gómez-Aldapa CA, Rangel-Vargas E, et al. Frequency of indicator bacteria, *Salmonella* and diarrhoeagenic *Escherichia coli* pathotypes on ready-to-eat cooked vegetable salads from Mexican restaurants. Lett Appl Microbiol 2013; 56(6): 414-420.
24. Gómez-Aldapa CA, Torres-Vitela Mdel R, Acevedo-Sandoval OA, et al. Presence of Shiga toxin–producing *Escherichia coli*, enteroinvasive *E. coli*, enteropathogenic *E. coli*, and enterotoxigenic *E. coli* on tomatoes from public markets in Mexico. J Food Prot 2013; 76(9): 1621-1625.
25. Paredes-Paredes M, Okhuysen PC, Flores J, et al. Seasonality of diarrheagenic *Escherichia coli* pathotypes in the US students acquiring diarrhea in Mexico. J Travel Med 2011; 18(2): 121-125.
26. Canizalez-Roman A, Flores-Villaseñor HM, Gonzalez-Nuñez E, et al. Surveillance of diarrheagenic *Escherichia coli* strains isolated from diarrhea cases from children, adults and elderly at Northwest of Mexico. Front Microbiol 2016; 7: 1924. doi:10.3389/fmicb. 2016.01924.
27. Amézquita-López BA, Quiñones B, Soto-Beltrán M, et al. Antimicrobial resistance profiles of Shiga toxin-producing *Escherichia coli* O157 and Non-O157 recovered from domestic farm animals in rural communities in Northwestern Mexico. Antimicrob Resist Infect Control 2016; 5: 1. doi:10.1186/s13756-015-0100-5.
28. Hasan MS, Yousif A, Alwan MJ. Detection of virulent genes in *E. coli* O157: H7 isolated from puppies and adult dogs by polymerase chain reaction. Res J Vet Pract 2016; 4(1): 1-6.
29. Meng Q, Bai X, Zhao A, et al. Characterization of Shiga toxin-producing *Escherichia coli* isolated from healthy pigs in China. BMC Microbiol 2014; 14: 5. doi:10.1186/1471-2180-14-5.
30. Buranasinsup S, Kulpeanprasit S, Kong-ngoen T, et al. Prevalence of the multi-drug resistance of Shiga toxin-producing *Escherichia coli* isolated from pigs in central Thailand. Chiang Mai J Sci 2018; 45(1): 21-32.
31. Samanta I, Joardar SN, Mahanti A, et al. Approaches to characterize extended spectrum beta-lactamase/beta-lactamase producing *Escherichia coli* in healthy organized vis-a-vis backyard farmed pigs in India. Infect Genet Evol 2015; 36: 224-230.
32. Changkaew K, Intarapuk A, Utrarachkij F, et al. Antimicrobial resistance, extended-spectrum β-lactamase productivity, and class 1 integrons in *Escherichia coli* from healthy swine. J Food Prot 2015; 78(8): 1442-1450.
33. Delmani FA, Jaran AS, Al Tarazi Y, et al. Characterization of ampicillin resistant gene (*blaTEM-1*) isolated from *E. coli* in Northern Jordan. Asian J Biomed Pharmaceut Sci 2017; 7(61): 11-15.
34. Nagachinta S, Chen J. Integron-mediated antibiotic resistance in Shiga toxin-producing *Escherichia coli*. J Food Prot 2009; 72(1): 21-27.