World Journal of Microbiology and Biotechnology (2019) 35:134
https://doi.org/10.1007/s11274-019-2712-z

ORIGINAL PAPER

Characterization of non-O157 Shiga toxin-producing *Escherichia coli* (STEC) obtained from feces of sheep in Brazil

João Pedro Rueda Furlan[1] · Inara Fernanda Lage Gallo[1] · Anna Carolina Leonelli Pires de Campos[2] · Armando Navarro[3] · Renata Katsuko Takayama Kobayashi[2] · Gerson Nakazato[2] · Eliana Guedes Stehling[1,4]

Received: 5 April 2019 / Accepted: 8 August 2019 / Published online: 20 August 2019

Abstract

Shiga toxin-producing *Escherichia coli* (STEC) are zoonotic pathogens and may induce severe diarrheagenic diseases in humans and other animals. Non-O157 STEC have been emerging as important pathogens causing outbreaks worldwide. Bacterial resistance to antimicrobials has become a global public health problem, which involves different ecological spheres, including animals. This study aimed to characterize the resistance to antimicrobials, plasmids and virulence, as well as the serotypes and phylogenetic groups in *E. coli* isolated from sheep in Brazil. A total of 57 isolates were obtained and showed different antimicrobial resistance profiles. Nineteen isolates presented acquired antimicrobial resistance genes (ARGs) (*bla*$_{\text{CTX-M-Gp9}}$, *qnrB, qnrS, oqxB, oqxA, tetA, tetB, tetC, sul1* and *sul2*) and plasmid families (F, FIA, FIB, I1, K, HI1 and ColE-*like*). The *stx*1, *stx*2 and *ehx*A virulence genes were detected by PCR, being 50 isolates (87.7%) classified as STEC. A great diversity of serotypes was detected, being O176:HNM the most predominant. Phylogenetic group E was the most prevalent, followed by B1, A and B2. To the best of our knowledge, this is the first report in the world of *bla*$_{\text{CTX-M-Gp9}}$ (O75, O114, O100, O128ac and O176 serogroups), *qnrB* and *oqxB* genes in non-O157 STEC in healthy sheep. The results obtained in the present study call attention to the monitoring of antimicrobial-resistant non-O157 STEC harboring acquired ARGs worldwide and indicate a zoonotic risk due to the profile of virulence, resistance and serotype found.

Keywords *Escherichia coli* · STEC · Resistance · CTX-M-*like* · Non-O157

Introduction

Shiga toxin-producing *Escherichia coli* (STEC) are zoonotic pathogens related to the hemolytic uremic syndrome (HUS) and hemorrhagic colitis. STEC isolates colonize the gastrointestinal tract of sheep and bovine without causing any disease; however, may induce severe diarrheagenic diseases in humans and other animals (Ferens and Hovde 2011; Kumar et al. 2012). Outbreaks of STEC have been reported worldwide since the STEC O157:H7 is responsible for the most serious outbreaks; however, non-O157 STEC has emerged as important pathogens (Folster et al. 2011; Etcheverría and Padola 2013).

Bacterial resistance to antimicrobials has become a global public health problem, which involves different ecological spheres, including animals. This concern occurs due to the emergence of multidrug-resistant (MDR) bacteria, including *E. coli*. Increased attention has been given and more studies involving animal sampling have taken place because animals act as reservoirs and disseminators of acquired antimicrobial

✉ Eliana Guedes Stehling
elianags@usp.br

[1] Departamento de Análises Clínicas, Toxicológicas e Bromatológicas, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo (USP), Ribeirão Preto, Brazil

[2] Departamento de Microbiologia, Centro de Ciências Biológicas, Universidade Estadual de Londrina (UEL), Londrina, Brazil

[3] Departamento de Salud Pública, Facultad de Medicina, Universidad Nacional Autônoma de México, Ciudad Universitaria, Mexico City, Mexico

[4] Present Address: Departamento de Análises Clínicas, Toxicológicas e Bromatológicas, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo (USP), Av. do Café S/N. Monte Alegre, Ribeirão Preto, SP 14040-903, Brazil

resistance genes (ARGs) (Salyers and Shoemaker 2006; Toner et al. 2015).

Antimicrobial-resistant STEC obtained from different sources, including animals, and belonging to different serotypes have been reported worldwide, which is worrying (Martin et al. 2015; Mukherjee et al. 2017). Therefore, this study aimed to characterize the resistance to antimicrobials, plasmids and virulence as well as the serotypes and phylogenetic groups in *E. coli* isolated from sheep in Brazil.

Materials and methods

Obtaining isolates

Fecal samples were collected between 2016 and 2017 from healthy sheep in two farms located in São Paulo State, Brazil. The farm A is 43 km away from Farm B with no reports of antimicrobials use in both farms. One gram of each fecal sample was added in 5 mL of sterile saline solution (0.9% NaCl). Then, the samples were seeded using disposable inoculating loops (10 µL) on MacConkey Agar (Oxoid, UK) and incubated at 37 °C for 18–24 h. Finally, the isolates with morphological characters of *E. coli* were selected and stocked at − 80 °C in Brain Heart Infusion broth (Oxoid, UK) with 15% glycerol for further identification by sequencing of the 16S rDNA gene.

Extraction of genomic DNA, PCR conditions and amplicon sequencing

Genomic DNA extraction was performed using the GenElute™ Bacterial Genomic DNA Kit (Sigma-Aldrich, USA) according to the manufacturer's recommendations. All PCR reactions were performed using the 1.25U (2 µL) of JumpStart™ Taq DNA Polymerase (Sigma-Aldrich, USA), 5 µL of 10X PCR buffer without MgCl2, 25 mM (3.5 µL) of MgCl2, 10 mmol L^{-1} (1 µL) of deoxynucleotide solution mix, 25 µM (2 µL) of each primer, 100 ng (5 µL) of DNA and 29.5 µL of ultrapure water, totalizing a reaction mixture of 50 µL on a ProFlex™ PCR Thermocycler System (Applied Biosystems, Singapore). Positive and negative controls were used in all PCR reactions.

The amplicons were purified using the Illustra™ GFX™ PCR DNA and Gel Band Purification Kit (GE Healthcare, UK). The sequencing was performed on an ABI 3500xL Genetic Analyzer (Applied Biosystems, USA) using the BigDye™ Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific, USA). The sequences were analyzed using tools available in GenBank (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Identification of isolates

The isolates were identified by sequencing of the 16S rDNA gene using the following primers: fD1 (5′-AGAGTTTGATCCTGGCTCAG-3′) and rP2 (5′-ACGGCTACCTTGTTACGACTT-3′) (Weisburg et al. 1991).

Antimicrobial susceptibility testing

The antimicrobial susceptibility testing was performed as recommended by the Clinical and Laboratory Standards Institute (CLSI 2017) using the disk diffusion method. A total 36 antimicrobials was tested for each isolate, including ampicillin, amoxicillin-clavulanate, ampicillin-sulbactam, piperacillin-tazobactam, ticarcillin-clavulanate, cefazolin, cefoxitin, cefuroxime, cefaclor, ceftazidime, ceftriaxone, cefotaxime, cefepime, aztreonam, ertapenem, imipenem, meropenem, gentamicin, tobramycin, amikacin, nalidixic acid, lomefloxacin, ofloxacin, ciprofloxacin, norfloxacin, levofloxacin, streptomycin, tetracycline, doxycycline, minocycline,, trimethoprim-sulfamethoxazole, sulfonamide, trimethoprim, chloramphenicol and nitrofurantoin. *E. coli* ATCC® 25922 and *Pseudomonas aeruginosa* ATCC® 27853 strains were used as control.

Detection of acquired ARGs

Acquired ARGs were screened by PCR in non-susceptible (intermediate or resistant) isolates for β-lactams ($bla_{\text{CTX-M}}$ groups 1, 2, 8 and 9, bla_{SHV}, bla_{CMY} and $bla_{\text{OXA-1-}like}$), tetracyclines (*tetA, tetB, tetC, tetD, tetE, tetG, tetJ, tetL, tetM, tetO, tetS, tetP, tetQ* and *tetX*), quinolone and fluoroquinolones (*qnrA, qnrB, qnrS, qepA, oqxA* and *oqxB*), aminoglycosides (*aac(3′)-Ia, aac(3′)-IIa, ant(2″)-Ia, aac(6′)-Ih, aph(3′)-VI, aac(6′)-Ib* and *aph(3′)-Ia*) and sulfonamides (*sul1, sul2* and *sul3*) (Noppe-Leclercq et al. 1999; Ng et al. 2001; Kerrn et al. 2002; Perreten and Boerlin 2003; Cattoir et al. 2007; Dallenne et al. 2010; Karczmarczyk et al. 2011; Chen et al. 2012).

Detection of virulence genes

Detection of the diarrheagenic virulence genes was performed by PCR. The genes *ipaH stx1, stx2, ehxA, aaiC, aatA, eaeA, bfpA, aggR, elt est, aap, aggR* and *AA probe* were detected using the primers described by Schmidt et al. (1995), Paton and Paton (1998), Aranda et al. (2007), Lima et al. (2013) and Cerna et al. (2003).

Plasmid replicon typing

Twenty plasmid families (IncHI2, IncHI1, IncI1, IncFIB, IncFIA, IncFIC, IncF, IncFIIA, IncL/M, IncW, IncP, IncA/C, IncK, IncN, IncY, IncT, IncX, IncU, IncR and ColE-*like*)

were researched by PCR-based replicon typing according to Carattoli et al. (2005) and García-Fernández et al. (2009).

Escherichia coli serotyping

Escherichia coli serotyping was performed using rabbit antisera against antigens [O1 to O187 somatic (O) and 53 flagellar (H)] by agglutination assays using 96-well Microtiter™ microplates (Thermo Scientific, USA) according to Orskov and Orskov (1984) and Scheutz et al. (2004).

Phylogenetic group determination

Phylogenetic groups (A, B1, B2, C, D, E and F) were determined using the phylo-typing method according to Clermont et al. (2013). The phylogenetic groups were determined using the quadruplex genotype (*arpA, chuA, yjaA, TspE4.C2*), being A (+ − −), B1 (+ − +), B2 (− + + −, − + + + or − + − +) and F (− + −). C- and E-specific primers (*trpA* and *arpA*) were used for classification of phylogenetic groups C (+ − + −, C+), D (+ +− or + + − +, E-) and E (+ +− or + + − +, E+).

Results

Isolates, genes and phylogenetic groups

In this study, 57 bacterial isolates were obtained, being 42 from farm A and 15 from farm B. These isolates showed different antimicrobial resistance profiles, several acquired ARGs and different plasmid families, including ColE-*like*, F, FIB, FIA, I1, K and HI1. The *stx1, stx2* and *ehxA* virulence genes were detected, being 50 isolates (87.7%) classified as STEC and a great diversity of serotypes was observed, being O176:HNM the most prevalent. Four phylogenetic groups were detected (A, B1, B2 and E) and four isolates (A4, A5, A6 and A27) were not classified into any phylogenetic group, being denominated as unknown (Tables 1, 2).

The results were divided into two groups for a better presentation. In the first one were reported the isolates that presented acquired ARGs, which also presented non-susceptibility for several antimicrobials and the presence of plasmids. In group 2 were gathered the isolates with no acquired ARGs, being some of them susceptible to all tested antimicrobials and other non-susceptible to at least one antimicrobial. The sequences obtained from the 16S rRNA and acquired ARGs were deposited in the GenBank (www.ncbi.nlm.nih.gov/Genbank) with accession numbers MK506922-MK506978, MK532862-MK532896, MK543886 and MK543887.

Characterization of Group 1

Group 1 has 19 isolates [Farm 1 (15) and Farm 2 (4)], being 11 (57.9%) non-susceptible to sulfonamide, nine (47.3%) to ampicillin, cefazolin, cefoxitin and cefaclor, seven (36.8%) to tetracycline and doxycycline, five (26.3%) to ciprofloxacin, levofloxacin, norfloxacin, lomefloxacin and ofloxacin, and four (21%) to trimethoprim-sulfamethoxazole, nalidixic acid, streptomycin and trimethoprim. A total of 38 amplicons from different acquired ARGs were detected and according to this profile, the $bla_{\text{CTX-M-Gp9}}$ (9) was the most prevalent, followed by *tetB* (6), *qnrS* (5), *tetA* (4), *qnrB* (4), *sul1* (2), *sul2* (2), *oqxB* (2), *oqxA* (2) and *tetC* (1) (Table 1).

Seven plasmid families were found in the group 1, being the ColE-*like* (12) the most prevalent, followed by FIB (7), F (6), I1 (4), K (4), FIA (1) and HI1 (1). The *stx*1 gene was detected in 12 isolates, followed by *ehxA* (11) and *stx2* (1) genes. Twelve isolates were classified as STEC. Thirteen serotypes were detected in this group, including O154:H9 (4), O176:HNM (3), O185:HNM (2), O70:HNM (1), O75:HNM (1), O86:H2 (1), O100:H21 (1), O114:H14 (1), O129:H20 (1), A128ac:H2 (1), O176:H19 (1), O185:H16 (1) and ONT:HNM (1). Seven isolates were classified into phylogenetic group B1, four into A and unknown, three into E and one into B2 (Table 1).

Characterization of Group 2

In group 2, 38 isolates were gathered [Farm 1 (27) and Farm 2 (11)]. Among them, 24 (63.1%) were non-susceptible to sulfonamide, 14 (36.8%) to cefazolin, 12 (31.5%) to cefaclor, three (7.9%) to ampicillin, and one (2.6%) to chloramphenicol, cefuroxime, gentamicin, tobramycin and amikacin. The *stx*1 gene was detected in all isolates, followed by *ehx*A (31) and *stx*2 (5) genes. Thus, all isolates were classified as STEC. The O176:HNM (24) serotype was the most prevalent, followed by O91:HNM (3); O75:HNM (2), O48:H16 (1), O88:H4 (1), O88:H25 (1), O114:H4 (1), O93:H52 (1), O139:H19 (1), O75:H8 (1), O8:H19 (1) and ONT:HNM (1) The majority of isolates was classified into phylogenetic group E (25), followed by B1 (11), A (1) and B2 (1) (Table 2). In general, it was observed that isolates belonging to the group 1 presented a higher resistance profile, while the group 2 showed a greater virulence potential, with all isolates classified as STEC since the *eaeA* gene was not detected.

Discussion

The majority of outbreaks caused by STEC are related to the consumption of contaminated products with animal feces, including sheep. STEC produces Shiga toxins 1 (Stx1) and

Table 1 Characteristics of non-O157 STEC that presented acquired ARGs

Isolate	Farm	Resistance profile[a]	Acquired ARGs	Virulence genes	Pathotype	Serotype[b]	Plasmid family	Phylogeneticgroup[c]
A3	1	STP, TET, DOX, SUL	*tetB*	*ehxA, stx1*	STEC	O70:HNM	K	Unknown
A4	1	TET, DOX, SUL	*tetA, tetB*	*ehxA, stx1*	STEC	ONT:HNM	FIB, K, ColE-*like*	Unknown
A5	1	AMP, CFZ, CFO, CFC, STP, TET, DOX, SUL	$bla_{\text{CTX-M-Gp9}}$, *tetB*	*ehxA, stx1*	STEC	O185:H16	ColE-*like*	Unknown
A12	1	AMP, CFZ, CFO, CFC, SUL	$bla_{\text{CTX-M-Gp9}}$	*ehxA, stx1*	STEC	O176:HNM	FIB, ColE-*like*	E
A16	1	AMP, CFZ, CFO, CFC	$bla_{\text{CTX-M-Gp9}}$	*ehxA, stx1*	STEC	O176:HNM	ColE-*like*	E
A17	1	AMP, CFZ, CFO, CFC	$bla_{\text{CTX-M-Gp9}}$	*ehxA, stx1*	STEC	O75:HNM	FIB, ColE-*like*	B1
A23	1	AMP, CFZ, CFO, CFC	$bla_{\text{CTX-M-Gp9}}$	–	–	O114:H14	FIB, ColE-*like*	B2
A25	1	AMP, CFZ, CFO, CFC, TET, DOX, SUL	$bla_{\text{CTX-M-Gp9}}$, *tetA, tetC*	–	–	O100:H21	F	B1
A27	1	CIP, LVX, NOR, LMX, OFX	*qnrS*	*stx1*	STEC	O129:H20	F, FIB, ColE-*like*	Unknown
A34	1	AMP, CFZ, CFO, CFC	$bla_{\text{CTX-M-Gp9}}$	*ehxA, stx1, stx2*	STEC	O128ac:H2	F, FIA, FIB, I1, K	B1
A38	1	TET, DOX	*tetA, tetB*	–	–	O86:H2	F	B1
A46	1	CIP, LVX, NOR, LMX, OFX, NAL, SXT, SUL, TRI	*sul2, qnrB, qnrS*	–	–	O154:H9	F, ColE-*like*	A
A47	1	CIP, LVX, NOR, LMX. OFX, NAL, SXT, SUL, TRI	*sul2, qnrB, qnrS*	–	–	O154:H9	F, ColE-*like*	A
A48	1	CIP, LVX, NOR, LMX, OFX, NAL, SXT, SUL, TRI	*sul1, qnrB, qnrS, oqxA, oqxB*	–	–	O154:H9	I1, ColE-*like*	A
A83	1	AMP, CFZ, CFC, CFO, SUL	$bla_{\text{CTX-M-Gp9}}$	*ehxA, stx1*	STEC	O176:HNM	HI1	B1
A50	2	CIP, LVX, NOR, LMX, OFX, NAL, SXT, SUL, TRI	*sul1, qnrB, qnrS, oqxA, oqxB*	–	–	O154:H9	I1, ColE-*like*	A
A54	2	STP, TET, DOX	*tet*A, *tet*B	*ehxA, stx1*	STEC	O185:HNM	FIB, K	B1
A55	2	AMP, CFZ, CFO, CFC	$bla_{\text{CTX-M-Gp9}}$	*ehxA, stx1*	STEC	O176:H19	F, ColE-*like*	E
A68	2	STP, TET, DOX, SUL	*tetB*	*ehxA, stx1*	STEC	O185:HNM	K	B1

[a]*STP* streptomycin, *TET* tetracycline, *DOX* doxycycline, *SUL* sulfonamide, *NAL* nalidixic acid, *SXT* trimethoprim-sulfamethoxazole, *TRI* trimethoprim, *CFC* cefaclor, *CFZ* cefazolin, *CFO* cefoxitin, *AMP* ampicillin, *CIP* ciprofloxacin, *LVX* levofloxacin, *NOR* norfloxacin, *LMX* lomefloxacina, *OFX* ofloxacina

[b]*NT* non-typeable, *HNM* nonmotile

[c]According to Clermont et al. (2013)

2 (Stx2), which are encoded by *stx1* and *stx2* genes, respectively. In addition, some virulence markers such as enterohemolysin (*ehxA*) may be associated with these toxins and some studies have reported that Stx2 is more commonly reported in cases of HUS (Paton and Paton 1998; Ferens and Hovde 2011; Kumar et al. 2012; Etcheverría and Padola 2013).

The Center for Disease Control and Prevention report (CDC 2015) showed an increase in the incidence rate of infections caused by non-O157 STEC between 1996 and 2015 in the United States, especially in children aged 1 to 4 years and Tseng et al. (2016) also reported a significant increase of non-O157 STEC cases over time. Among the large diversity of reported non-O157 STEC serotypes are

Table 2 Characteristics of non-O157 STEC susceptible and non-susceptible to at least one antimicrobial tested

Isolate	Farm	Resistance profile[a]	Virulence genes	Pathotype	Serotype[b]	Phylogenetic group[c]
A2	1	STP, SUL	*stx1, stx2*	STEC	O88:H4	A
A13	1	CFZ, CFC	*ehxA, stx1*	STEC	O114:H4	E
A18	1	SUL	*ehxA, stx1*	STEC	O176:HNM	E
A19	1	–	*ehxA, stx1*	STEC	O176:HNM	E
A20	1	–	*ehxA, stx1*	STEC	O176:HNM	E
A21	1	–	*stx1*	STEC	O91:HNM	B1
A22	1	CFZ, CRX	*ehxA, stx1*	STEC	O176:HNM	E
A28	1	–	*stx1*	STEC	O88:H25	E
A30	1	CFZ, SUL	*ehxA, stx1, stx2*	STEC	O176:HNM	E
A33	1	CFC	*ehxA, stx1*	STEC	O176:HNM	E
A35	1	–	*stx1*	STEC	O48:H16	E
A37	1	–	*stx1*	STEC	O93:H52	B1
A40	1	CFZ	*ehxA, stx1*	STEC	O176:HNM	B1
A42	1	CFZ	*ehxA, stx1, stx2*	STEC	O75:H8	B1
A82	1	CFC, SUL	*ehxA, stx1*	STEC	O176:HNM	E
A84	1	AMP, SUL	*ehxA, stx1*	STEC	ONT:HNM	B2
A86	1	CFZ, CFC, GEN, TOB, AMI, SUL	*ehxA, stx1*	STEC	O176:HNM	E
A87	1	CFZ, CFC, SUL	*ehxA, stx1*	STEC	O176:HNM	E
A88	1	SUL	*ehxA, stx1*	STEC	O176:HNM	E
A89	1	CFZ, SUL	*ehxA, stx1*	STEC	O176:HNM	E
A90	1	AMP, CFC, SUL	*ehxA, stx1*	STEC	O176:HNM	B1
A93	1	SUL	*ehxA, stx1*	STEC	O75:HNM	E
A94	1	CFZ, CFO, CFC, SUL	*ehxA, stx1*	STEC	O176:HNM	B1
A96	1	CFC, SUL	*ehxA, stx1*	STEC	O176:HNM	E
A97	1	SUL	*ehxA, stx1*	STEC	O8:H19	B1
A98	1	CFZ, SUL	*ehxA, stx1*	STEC	O176:HNM	E
A99	1	CFZ, SUL	*ehxA, stx1*	STEC	O176:HNM	B1
A53	2	–	*ehxA, stx1*	STEC	O139:HNM	E
A56	2	CFC	*ehxA, stx1*	STEC	O176:HNM	E
A57	2	AMP, CFC	*ehxA, stx1*	STEC	O176:HNM	E
A60	2	–	*ehxA, stx1*	STEC	O75:HNM	B1
A66	2	SUL	*stx1, stx2*	STEC	O91:HNM	B1
A69	2	SUL	*ehxA, stx1*	STEC	O176:HNM	E
A70	2	CFZ, SUL	*stx1, stx2*	STEC	O91:HNM	B1
A73	2	SUL	*ehxA, stx1*	STEC	O176:HNM	E
A74	2	SUL	*ehxA, stx1*	STEC	O176:HNM	E
A75	2	CFZ, CFC, SUL, TRI, CLO	*ehxA, stx1*	STEC	O176:HNM	E
A79	2	CFZ, CFC, SUL, SUL	*ehxA, stx1*	STEC	O176:HNM	E

[a]*STP* streptomycin, *SUL* sulfonamide, *CFC* cefaclor, *CFZ* cefazolin, *CRX* cefuroxime, *AMP* ampicillin, *GEN* gentamicin, *TOB* tobramycin, *AMI* amikacin, *CLO* chloramphenicol

[b]*NT* non-typeable, *HNM* nonmotile

[c]According to Clermont et al. (2013)

O8, O70, O75, O86, O88, O91, O93, O100, O114, O128, O129, O154 and O185, which are the same reported in the present study.

Amézquita-López et al. (2016), Kusumoto et al. (2016) and Ghanbarpour and Kiani (2013) reported non-O157 STEC obtained from animals, including sheep, which were non-susceptible to several antimicrobials, such as β-lactams, tetracyclines, aminoglycosides, fluoroquinolones and sulfonamides. In Brazil, similar results were also reported with strains collected from animals and humans (Cergole-Novella et al. 2011).

Many studies have characterized the phenotypic profile of antimicrobial resistance in non-O157 STEC isolates; however, there are few reports of acquired ARGs. β-lactamases belonging to CTX-M-group stand out among ESBLs because of their spectrum of action and dissemination in isolates from a variety of sources, including animals (Cantón et al. 2012). Some non-O157 STEC have already been detected carrying $bla_{\text{CTX-M-}}$group in serogroups not detected in this work, such as O5, O104, O111 and O145 (Valat et al. 2012; Ewers et al. 2014; Ferdous et al. 2016). However, this is the first report of $bla_{\text{CTX-M-Gp9}}$ in non-O157 STEC belonging to O75, O114, O100, O128ac and O176 serogroups.

There are few descriptions of non-O157 STEC carrying multiple acquired ARGs worldwide. Bai et al. (2016), Ferdous et al. (2016) and Srinivasa et al. (2011) reported a diversity of non-O157 STEC carrying acquired resistance genes for fluoroquinolones (*qnrS* and *oqxA*), tetracyclines (*tetA, tetB, tetC, tetM*) and sulfonamides (*sul1* and *sul2*) associated with different plasmid families, but there are no reports of *qnrB* and *oqxB* in non-O157 STEC.

Some plasmid families are correlated with the presence of acquired ARGs in STEC as well as in non-STEC, including those detected in the present study (Carattoli 2013; Ewers et al. 2014). Among the plasmid families, ColE-*like* and IncF are prevalent and have been detected worldwide carrying $bla_{\text{CTX-M}}$-groups, plasmid-mediated quinolone resistance genes (PMQR), *tet* genes and *sul* genes (Pallecchi et al. 2010; Carattoli 2013; Lyimo et al. 2016; Yang et al. 2015).

A diversity of phylogenetic groups was detected (A, B1, B2 and E) in STEC isolates resistant and susceptible to antimicrobials obtained of two Brazilian farms, including that classified as unknown. Stoppe et al. (2017) showed that there is no correlation between geographic location, date or feeding habits and phylogenetic groups in isolates obtained worldwide, even in Brazil. Mora et al. (2012) and Carlos et al. (2010) reported that phylogenetic group B1 was the most prevalent in STEC and non-STEC isolates from different sources, including sheep, and that phylogenetic groups A and B2 were also reported. Interesting, the majority of STEC isolates from the present study was classified into phylogenetic group E, which is probably less reported due to its recent identification (Clermont et al. 2013).

The results found in the present study call attention to the monitoring of antimicrobial-resistant non-O157 STEC harboring acquired ARGs worldwide. These isolates can spread to different sources, as humans and animals, and disseminate ARGs through horizontal gene transfer to other pathogens. To the best of our knowledge, this is the first report in the world of $bla_{\text{CTX-M-Gp9}}$ (O75, O114, O100, O128ac and O176 serogroups), *qnrB* and *oqxB* genes in non-O157 STEC in healthy sheep. The results indicate a zoonotic risk due to the profile of virulence, resistance and serotype found.

Acknowledgements The authors thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for fellowship [Grant No. 88882.180855/2018-01].

Funding This work was supported by São Paulo Research Foundation—FAPESP [Grant No. 2018/19539-0].

Compliance with ethical standards

Conflict of interest The authors have no conflicts of interest to declare.

References

Amézquita-López BA, Quiñones B, Soto-Beltrán M, Lee BG, Yambao JC, Lugo-Melchor OY, Chaidez C (2016) 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 5:1. https://doi.org/10.1186/s13756-015-0100-5

Aranda K, Fabbricotti SH, Fagundes-Neto U, Scaletsky ICA (2007) Single multiplex assay to identify simultaneously enteropathogenic, enteroaggregative, enterotoxigenic, enteroinvasive and Shiga toxin producing *Escherichia coli* strains in Brazilian children. FEMS Microbiol Lett 267:145. https://doi.org/10.1111/j.1574-6968.2006.00580.x

Bai L, Hurley D, Li J, Meng Q, Wang J, Fanning S, Xiong Y (2016) Characterisation of multidrug-resistant Shiga toxin-producing *Escherichia coli* cultured from pigs in China: co-occurrence of extended-spectrum β-lactamase- and mcr-1-encoding genes on plasmids. Int J Antimicrob Agents 48:445–448. https://doi.org/10.1016/j.ijantimicag.2016.06.021

Cantón R, González-Alba JM, Galán JC (2012) CTX-M enzymes: origin and diffusion. Front Microbiol 3:110. https://doi.org/10.3389/fmicb.2012.00110

Carattoli A (2013) Plasmids and the spread of resistance. Int J Med Microbiol 303(6–7):298–304. https://doi.org/10.1016/j.ijmm.2013.02.001

Carattoli A, Bertini A, Villa L, Falbo V, Hopkins KL, Threlfall EJ (2005) Identification of plasmids by PCR-based replicon typing. J Microbiol Methods 63:219–228. https://doi.org/10.1016/j.mimet.2005.03.018

Carlos C, Pires MM, Stoppe NC, Hachich EM, Sato MI, Gomes TA, Amaral LA, Ottoboni LM (2010) *Escherichia coli* phylogenetic group determination and its application in the identification of the major animal source of fecal contamination. BMC Microbiol 10:161. https://doi.org/10.1186/1471-2180-10-161

Cattoir V, Poirel L, Rotimi V, Soussy CJ, Nordmann P (2007) Multiplex PCR for detection of plasmid-mediated quinolone resistance qnr genes in ESBL-producing enterobacterial isolates. J Antimicrob Chemother 60:394–397. https://doi.org/10.1093/jac/dkm204

Centers for Disease Control and Prevention (CDC). National STEC Surveillance Annual Report, 2015. Atlanta, Georgia: US Department of Health and Human Services, CDC, 2017. Retrieved from https://www.cdc.gov/nationalsurveillance/pdfs/STEC_Annual_Summary_2015-508c.pdf. Accessed 10 Jan 2019.

Cergole-Novella MC, Pignatari AC, Castanheira M, Guth BE (2011) Molecular typing of antimicrobial-resistant Shiga-toxin-producing *Escherichia coli* strains (STEC) in Brazil. Res Microbiol 162:117–123. https://doi.org/10.1016/j.resmic.2010.09.022

Cerna JF, Nataro JP, Estrada-Garcia T (2003) Multiplex PCR for detection of three plasmid-borne genes of enteroaggregative *Escherichia coli* strains. J Clin Microbiol 41:2138–2140

Chen X, Zhang W, Pan W, Yin J, Pan Z, Gao S, Jiao X (2012) Prevalence of *qnr*, *aac(6')-Ib-cr*, *qep*A, and *oqx*AB in *Escherichia coli* isolates from humans, animals, and the environment. Antimicrob Agents Chemother 56:3423–3427. https://doi.org/10.1128/AAC.06191-11

Clermont O, Christenson JK, Denamur E, Gordon DM (2013) The Clermont *Escherichia coli* phylo-typing method revisited: improvement of specificity and detection of new phylo-groups. Environ Microbiol Rep 5:58–65. https://doi.org/10.1111/1758-2229.12019

CLSI (2017) Performance standards for antimicrobial susceptibility testing: twenty-seventh informational supplement. CLSI document M100–S27. Clinical and Laboratory Standards Institute, Wayne, PA

Dallenne C, Costa A, Decré D, Favier C, Arlet G (2010) Development of a set of multiplex PCR assays for the detection of genes enconding importante beta-lactamases in *Enterobacteriaceae*. J Antimicrob Chemother 65:490–495. https://doi.org/10.1093/jac/dkp498

Etcheverría AI, Padola NL (2013) Shiga toxin-producing *Escherichia coli*: factors involved in virulence and cattle colonization. Virulence 4:366–372. https://doi.org/10.4161/viru.24642

Ewers C, Stamm I, Stolle I, Guenther S, Kopp PA, Fruth A, Wieler LH, Scheufen S, Bauerfeind R, Bethe A, Prenger-Berninghoff E (2014) Detection of Shiga toxin- and extended-spectrum β-lactamase-producing *Escherichia coli* O145:NM and Ont:NM from calves with diarrhoea. J Antimicrob Chemother 69:2005–2007. https://doi.org/10.1093/jac/dku042

Ferdous M, Friedrich AW, Grundmann H, de Boer RF, Croughs PD, Islam MA, Kluytmans-van den Bergh MF, Kooistra-Smid AM, Rossen JW (2016) Molecular characterization and phylogeny of Shiga toxin-producing *Escherichia coli* isolates obtained from two Dutch regions using whole genome sequencing. Clin Microbiol Infect 22:642.e1–9. https://doi.org/10.1016/j.cmi.2016.03.028

Ferens WA, Hovde CJ (2011) *Escherichia coli* O157:H7: animal reservoir and sources of human infection. Foodborne Pathog Dis 8:465–487. https://doi.org/10.1089/fpd.2010.0673

Folster JP, Pecic G, Taylor E, Whichard J (2011) Characterization of Isolates from an outbreak of multidrug-resistant, Shiga toxin-producing *Escherichia coli* O145 in the United States. Antimicrob Agents Chemother 55:5955–5956. https://doi.org/10.1128/AAC.05545-11

García-Fernández A, Fortini D, Veldman K, Mevius D, Carattoli A (2009) Characterization of plasmids harbouring *qnr*S1, *qnr*B2 and *qnr*B19 genes in *Salmonella*. J Antimicrob Chemother 63:274–281. https://doi.org/10.1093/jac/dkn470

Ghanbarpour R, Kiani M (2013) Characterization of non-O157 shiga toxin-producing *Escherichia coli* isolates from healthy fat-tailed sheep in southeastern of Iran. Trop Anim Health Prod 45:641–648. https://doi.org/10.1007/s11250-012-0271-5

Karczmarczyk M, Abbott Y, Walsh C, Leonard N, Fanning S (2011) Characterization of multidrug-resistant *Escherichia coli* isolates from animals presenting at a university veterinary hospital. Appl Environ Microbiol 77:7104–7112. https://doi.org/10.1128/AEM.00599-11

Kerrn MB, Klemmensen T, Frimodt-Møller N, Espersen F (2002) Susceptibility of Danish *Escherichia coli* strains isolated from urinary tract infections and bacteraemia, and distribution of *sul* genes conferring sulphonamide resistance. J Antimicrob Chemother 50:513–516. https://doi.org/10.1093/jac/dkf164

Kumar A, Taneja N, Kumar Y, Sharma M (2012) Detection of Shiga toxin variants among Shiga toxin-forming *Escherichia coli* isolates from animal stool, meat and human stool samples in India. J Appl Microbiol 113:1208–1216. https://doi.org/10.1111/j.1365-2672.2012.05415.x

Kusumoto M, Hikoda Y, Fujii Y, Murata M, Miyoshi H, Ogura Y, Gotoh Y, Iwata T, Hayashi T, Akiba M (2016) Emergence of a multidrug-resistant Shiga toxin-producing enterotoxigenic *Escherichia coli* lineage in diseased swine in Japan. J Clin Microbiol 54:1074–1081. https://doi.org/10.1128/JCM.03141-15

Lima IF, Boisen N, Quetz Jda S, Havt A, de Carvalho EB, Soares AM, Lima NL, Mota RM, Nataro JP, Guerrant RL, Lima AÂ (2013) Prevalence of enteroaggregative *Escherichia coli* and its virulence-related genes in a case-control study among children from north-eastern Brazil. J Med Microbiol 62:683–693. https://doi.org/10.1099/jmm.0.054262-0

Lyimo B, Buza J, Subbiah M, Temba S, Kipasika H, Smith W, Call DR (2016) IncF plasmids are commonly carried by antibiotic resistant *Escherichia coli* isolated from drinking water sources in Northern Tanzania. J Med Microbiol 2016:3103672. https://doi.org/10.1155/2016/3103672

Martin MJ, Thottathil SE, Newman TB (2015) Antibiotics overuse in animal agriculture: a call to action for health care providers. Am J Public Health 105:2409–2410. https://doi.org/10.2105/AJPH.2015.302870

Mora A, López C, Dhabi G, López-Beceiro AM, Fidalgo LE, Díaz EA, Martínez-Carrasco C, Mamani R, Herrera A, Blanco JE, Blanco M, Blanco J (2012) Seropathotypes, phylogroups, Stx subtypes, and intimin types of wildlife-carried, shiga toxin-producing *Escherichia coli* strains with the same characteristics as human-pathogenic isolates. Appl Environ Microbiol 78:2578–2585. https://doi.org/10.1128/AEM.07520-11

Mukherjee S, Mosci RE, Anderson CM, Snyder BA, Collins J, Rudrik JT, Manning SD (2017) Antimicrobial drug-resistant Shiga toxin-producing *Escherichia coli* infections, Michigan, USA. Emerg Infect Dis 23:1609–1611. https://doi.org/10.3201/eid2309.170523

Ng LK, Martin I, Alfa M, Mulvey M (2001) Multiplex PCR for the detection of tetracycline resistant genes. Mol Cell Probes 15:209–215. https://doi.org/10.1006/mcpr.2001.0363

Noppe-Leclercq I, Wallet F, Haentjens S, Courcol R, Simonet M (1999) PCR detection of aminoglycoside resistance genes: a rapid molecular typing method for *Acinetobacter baumannii*. Res Microbiol 150:317–322

Orskov F, Orskov I (1984) Serotyping of *Escherichia coli*. Methods Microbiol 14:43–112

Pallecchi L, Riccobono E, Sennati S, Mantella A, Bartalesi F, Trigoso C, Gotuzzo E, Bartoloni A, Rossolini GM (2010) Characterization of small ColE-like plasmids mediating widespread dissemination of the *qnr*B19 gene in commensal enterobacteria. Antimicrob Agents Chemother 54:678–682. https://doi.org/10.1128/AAC.01160-09

Paton AW, Paton JC (1998) Detection and characterization of Shiga toxigenic *Escherichia coli* by using multiplex PCR assays for *stx*1, *stx*2, *eae*A, enterohemorrhagic *E. coli hly*A, rfbO111, and rfbO157. J Clin Microbiol 36:598–602

Perreten V, Boerlin P (2003) A new sulfonamide resistance gene (*sul*3) in *Escherichia coli* is widespread in the pig population of Switzerland. Antimicrob Agents Chemother 47:1169–1172

Salyers A, Shoemaker NB (2006) Reservoirs of antibiotic resistance genes. Anim Biotechnol 17:137–146. https://doi.org/10.1080/10495390600957076

Scheutz F, Cheasty T, Woodward D, Smith HR (2004) Designation of O174 and O175 to temporary O groups OX3 and OX7, and six new *E. coli* O groups that include Verocytotoxin-producing *E. coli* (VTEC): O176, O177, O178, O179, O180 and O181. APMIS 112:569–584. https://doi.org/10.1111/j.1600-0463.2004.apm11120903.x

Schmidt H, Knop C, Franke S, Aleksic S, Heesemann J, Karch H (1995) Development of PCR for screening of enteroaggregative *Escherichia coli*. J Clin Microbiol 33:701–705

Srinivasa TR, Gill JPS, Kumar GVVPS, Ghatak S (2011) Multi drug resistance patterns of Shiga toxin–producing *Escherichia coli* (STEC) and non–STEC isolates from meats, RTE meat foods,

drinking water and human diarrhoeic samples of Punjab. India Arch Clin Microbiol 2:3. https://doi.org/10.3823/225

Stoppe NC, Silva JS, Carlos C, Sato MIZ, Saraiva AM, Ottoboni LMM, Torres TT (2017) Worldwide phylogenetic group patterns of *Escherichia coli* from commensal human and wastewater treatment plant isolates. Front Microbiol 8:2512. https://doi.org/10.3389/fmicb.2017.02512

Toner E, Adalja A, Gronvall GK, Cicero A, Inglesby TV (2015) Antimicrobial resistance is a global health emergency. Health Secur 13:153–155. https://doi.org/10.1089/hs.2014.0088

Tseng M, Sha Q, Rudrik JT, Collins J, Henderson T, Funk JA, Manning SD (2016) Increasing incidence of non-O157 Shiga toxin-producing *Escherichia coli* (STEC) in Michigan and association with clinical illness. Epidemiol Infect 144:1394–1405. https://doi.org/10.1017/S0950268815002836

Valat C, Haenni M, Saras E, Auvray F, Forest K, Oswald E, Madec JY (2012) CTX-M-15 extended-spectrum β-lactamase in a shiga toxin-producing *Escherichia coli* isolate of serotype O111:H8. Appl Environ Microbiol 78:8–9. https://doi.org/10.1128/AEM.06997-11

Weisburg WG, Barns SM, Pelletier BA, Lane DJ (1991) 16S ribosomal DNA amplification for phylogenetic study. J Bacteriol 173:697–703. https://doi.org/10.1128/jb.173.2.697-703.1991

Yang QE, Sun J, Li L, Deng H, Liu BT, Fang LX, Liao XP, Liu YH (2015) IncF plasmid diversity in multi-drug resistant *Escherichia coli* strains from animals in China. Front Microbiol 6:964. https://doi.org/10.3389/fmicb.2015.00964