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Stx1 and *Stx2* subtyping and antimicrobial resistance in Shiga toxin-producing *Escherichia coli* (STEC) isolates from cattle and sheep feces in the Southeastern region of the State of Goiás, Brazil[1]

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ABSTRACT.- Arrais B.R., Silveira A.V.B.A., Oliveira A.F., Barbosa N.C., Stella A.E., Alves B.G., Ferreira M.R.A. & Moreira C.N. 2021. ***Stx1* and *Stx2* subtyping and antimicrobial resistance in Shiga toxin-producing *Escherichia coli* (STEC) isolates from cattle and sheep feces in the Southeastern region of the State of Goiás, Brazil**. *Pesquisa Veterinária Brasileira 41:e06747, 2021*. Graduate Program in Animal Bioscience, Universidade Federal de Jataí, Campus Jatobá, BR-364 Km 195 3800, Cidade Universitária, Jataí, GO 75801-615, Brazil. E-mail: marcosferreiravet@gmail.com

The present study was aimed at subtyping of *Stx1* and *Stx2* genes and characterization of antimicrobial resistance in 106 Shiga toxin-producing *Escherichia coli* (STEC) strains isolated from cattle and sheep feces. PCR was used to determine the subtypes, and the disk-diffusion method was used to evaluate the antimicrobial resistance. Ten antibiotics from five different classes were tested. Among the isolates of bovine origin, two subtypes of *Stx1* (*Stx1a* and *Stx1c*), and four subtypes of *Stx2* (*Stx2a*, *Stx2b*, *Stx2c,* and *Stx2d*) were identified. In isolates of sheep origin, two subtypes of *Stx1* (*Stx1a* and *Stx1c*), and four subtypes of *Stx2* (*Stx2a*, *Stx2b*, *Stx2c,* and *Stx2 g*) were identified. The results obtained suggest the presence of high diversity in *Stx1* and *Stx2* genes. Further, 96.6% (57/59) of bovine fecal strains and 89.4% (42/47) of sheep fecal strains showed resistance to at least one tested antibiotic. In both animal species, most strains were multidrug-resistant (MDR) (67.8% in cattle and 59.6% in sheep), with no significant difference between host animals. Adult animals were eight times more likely to have STEC with greater pathogenic potential. STEC with the highest pathogenic potential were three times more likely to be multidrug-resistant than STEC with the lowest pathogenic potential. The data reported in this study suggests the occurrence of strains with high potential pathogenicity in the region studied. Therefore, the ruminants of this region are carriers of strains that can cause infections in humans.

INDEX TERMS: *Stx1*, *Stx2*, subtyping, antimicrobial resistance, Shiga toxin, *Escherichia coli*, STEC, cattle, sheep, feces, Brazil, ruminants, public health, food security, multidrug-resistant, virulence factors.

RESUMO.- [**Subtipagem de *Stx1* e *Stx2* e resistência antimicrobiana em isolados de *Escherichia coli* produtoras de toxina Shiga (STEC) de fezes de bovinos e ovinos na região sudeste de Goiás, Brasil.**] O presente estudo teve como objetivo subtipar os genes *Stx1* e *Stx2* e caracterizar a resistência antimicrobiana em 106 isolados de *Escherichia coli* produtoras de toxinas Shiga (STEC) isoladas de fezes de bovinos e ovinos. A PCR foi utilizada para determinar os subtipos e o método de difusão em disco foi utilizado para avaliar a resistência antimicrobiana. Dez antibióticos de cinco classes diferentes foram testados. Entre os isolados de origem bovina, foram identificados dois subtipos de *Stx1* (*Stx1a* e *Stx1c*) e quatro subtipos de Stx2 (*Stx2a*, *Stx2b*, *Stx2c* e *Stx2d*). Nos isolados de origem ovina, foram identificados dois subtipos de Stx1 (Stx1a e Stx1c) e quatro subtipos de *Stx2* (*Stx2a*, *Stx2b*, *Stx2c* e *Stx2g*). Os resultados obtidos sugerem

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a presença de alta variabilidade nos genes *Stx1* e *Stx2*. Além disso, 96,6% (57/59) dos isolados fecais de bovinos e 89,4% (42/47) dos isolados de ovinos mostraram resistência a pelo menos um antibiótico testado. Em ambas as espécies animais, a maioria das cepas foi multirresistente (MDR) (67,8% em bovinos e 59,6% em ovinos), sem diferença significativa entre as espécies animais do reservatório. Os animais adultos tiveram oito vezes mais chances de apresentar STEC com maior potencial patogênico. STEC com o maior potencial patogênico teve três vezes mais chances de ser multirresistente do que o STEC com o menor potencial patogênico. Os dados relatados neste estudo sugerem a ocorrência de cepas com alto potencial de patogenicidade na região estudada. Portanto, os ruminantes dessa região são hospedeiros de isolados que podem causar infecções em humanos.

TERMOS DE INDEXAÇÃO: Subtipagem, *Stx1*, *Stx2*, resistência antimicrobiana, *Escherichia coli*, toxina Shiga, STEC, fezes, bovinos, ovinos, Brasil, ruminantes, saúde pública, segurança alimentar, mutidroga resitentes, fatores de virulência.

INTRODUCTION

Shiga toxin-producing *Escherichia coli* (STEC) strains are associated with serious infections in humans, such as hemolytic uremic syndrome (HUS), hemorrhagic colitis, meningitis, and septicemia (Karmali et al. 2010). They pose a serious public health threat, as they are frequently associated with severe illness and outbreaks in humans, which are transmitted from animals (Veneti et al. 2019). STEC is a diverse group of bacteria characterized by the production of potent cytotoxins, called Shiga toxin 1 and Shiga toxin 2 (Stx1 and Stx2), which bind to the same receptor and act on the same target in the cell but differ in the level of cytotoxicity (Russo et al. 2014, Cherubin et al. 2019). Ruminants, especially cattle and sheep, are the main reservoirs of STEC, shedding these bacteria in the feces, which get directly or indirectly transmitted to humans, thereby causing diseases (Ferreira et al. 2014, Yang et al. 2017).

There are three subtypes of *Stx1* (*Stx1a*, *Stx1c,* and *Stx1d*) and seven of *Stx2* (*Stx2a*, *Stx2b*, *Stx2c, Stx2d, Stx2e, Stx2f,* and *Stx2g*). The simultaneous expression of more than one subtype can increase the in-vivo toxicity of the non-O157 strain, making it as virulent as the O157: H7 strain (Scheutz et al. 2012, Jajarmi et al. 2017). *Stx1a* is more cytotoxic to Vero cells than *Stx2a*, but *Stx2a* has a lower 50% lethal dose (LD_{50}) in mice (Russo et al. 2014, Cherubin et al. 2019). Epidemiological data suggest that infections with STEC isolates that produce only *Stx2a*, progress more frequently to HUS than isolates that produce only *Stx1a* or produce both *Stx1a* and *Stx2a* (Petro et al. 2019).

STEC strain has shown markedly high resistance to antimicrobials. The development of multidrug-resistant (MDR) strains contributes to the increase of emerging pathogens and facilitates the movement of mobile genetic elements of drug resistance, leading to the spread of antibiotic resistance to other bacteria (Gentle et al. 2020, Yang et al. 2020). The objective of the present study was to identify the subtypes in 106 STEC strains isolated from cattle and sheep feces, to determine the susceptibility of these subtypes to antimicrobials, and to characterize them based on age, species, and sex of the animals.

MATERIALS AND METHODS

One hundred and six isolates STEC were selected from the collection of isolates of the "*Laboratório de Microbiologia Veterinária*" of the "Universidade Federal de Jataí" (UFJ). These were isolated from Girolando cattle (n=59) and healthy Santa Inês sheep (n=47) in studies carried out in the southwest region of the state of Goiás, Brazil (Ferreira et al. 2014, 2015). The isolates were selected according to age (young or adult), sex (males or females), and subtypes, i.e., *Stx1* or *Stx2* (Table 1).

For subtyping, the methodology and all the primers described by Scheutz et al. (2012) were used with adaptations. Triplex PCR for *Stx1* subtyping was performed in 25µL reaction mixture containing 5µL of the bacterial DNA, 1× PCR buffer (Sinapse Biotecnologia, Brazil), 3mM $MgCl_2$ (Sinapse Biotecnologia, Brazil), 0.4mM of dNTPs (Sinapse Biotecnologia, Brazil), 1U of Taq DNA polymerase (Sinapse Biotecnologia, Brazil), 0.2µM of each of the *Stx1c* and *Stx1d* primer pairs (Sigma-Aldrich, United States), and 0.4µM of *Stx1a* primer pair (Sigma-Aldrich, United States). The reactions for subtyping of *Stx2* samples were carried out in 20µL mixture containing 5µL of the bacterial DNA, 1 × PCR buffer (Sinapse Biotecnologia, Brazil), 3mM $MgCl_2$ (Sinapse Biotecnologia, Brazil), 0.4mM dNTP (Sinapse Biotecnologia, Brazil), 1U of Taq DNA polymerase (Sinapse Biotecnologia, Brazil), and 0.3µM of each primer pair.

All reactions were performed in a thermocycler (Veriti Thermal Cicler, Applied Biosystems, United States) programmed for initial denaturation at 95°C (15 min), followed by 35 cycles at 94°C (50 s), 64°C (40 s), 72°C (1 min), and a final extension at 72°C (3 min), except for those using *Stx2d* primers, when the annealing temperature was adjusted to 66°C. The amplified products were detected by 2% agarose gel electrophoresis (BioAmerica Biotech®). Electrophoretic separation was performed at 80 V for 2.5 h.

Antibiotic sensitivity tests were performed using the disk diffusion method according to the Clinical & Laboratory Standards Institute,

Table 1. Primers used to determine the subtypes of *Stx1* and *Stx2*

Primers	Sequences (5'-3')	Amplicon (bp)
stx1a-F1	CCTTTCCAGGTACAACAGCGGTT	478
stx1a-R2	GGAAACTCATCAGATGCCATTCTGG	
stx1c-F1	CCTTTCCTGGTACAACTGCGGTT	252
stx1c-R1	CAAGTGTTGTACGAAATCCCCTCTGA	
stx1d-F1	CAGTTAATGCGATTGCTAAGGAGTTTACC	203
stx1d-R2	CTCTTCCTCTGGTTCTAACCCCATGATA	
stx2a-F2	GCGATACTG**R**G**B**ACTGTGGCC*	349
stx2a-R3	CCG**K**CAACCTTCACTGTAAATGTG*	
stx2b-F1	AAATATGAAGAAGATATTTGTAGCGGC	251
stx2b-R1	CAGCAAATCCTGAACCTGACG	
stx2c-F1	GAAAGTCACAGTTTTTATATACAACGGGTA	177
stx2c-R2	CCGGCCACYTTTACTGTGAATGTA	
stx2d-F1	AAARTCACAGTCTTTATATACAACGGGTG	280
stx2d-R1	GCCTGATGCACAGGTACTGGAC	
stx2e-F1	CGGAGTATCGGGGAGAGGC	411
stx2e-R2	CTTCCTGACACCTTCACAGTAAAGGT	
stx2f-F1	TGGGCGTCATTCACTGGTTG	424
stx2f-R1	TAATGGCCGCCCTGTCTCC	
stx2g-F1	CACCGGGTAGTTATATTTCTGTGGATATC	573
stx2g-R1	GATGGCAATTCAGAATAACCGCT	

* Degenerate primers that were synthesized according to Scheutz et al. (2012).

with modifications (CLSI 2018). Ten antimicrobials of five classes were tested: β-Lactams (Ampicillin 10µg - AMP, Cefazolin 30µg - CFZ, Cefoxitin 30µg - CFO, Cefotaxime 30µg - CTX, Cefepime 30µg - CPM, Imipenem 10µg - IMP); Aminoglycosides (Streptomycin 10µg - EST); Quinolones (Ciprofloxacin 25µg - CIP); Sulfonamides (Sulfazotrim 25µg - STX); and Nitrofuran derivatives (Nitrofurantoin 300µg - NIT). *Escherichia coli* ATCC 25922 was used as a control strain (CLSI 2018). The isolates that had resistance to two or more different classes of antibiotics were considered MDR-isolates, according to Shaheen et al. (2010).

All statistical analyses were performed using the Sigma Plot version 11 program (Systat Software Inc., USA). The proportionate data were compared using the chi-square test. The logistic regression analysis evaluated the influence of age (young or adult), sex (male or female), species (bovine or sheep), and resistance (non-resistant or multi-resistant) as independent variables. The subtype presented was STEC with less pathogenic potential, i.e., subtypes of *Stx1*; and STEC with greater pathogenic potential, i.e., subtypes of *Stx2* or subtypes of both *Stx1* and *Stx2* (dependent variable). The generated data show the number of positive isolates for the *Stx* subtypes and were considered significant when $P<0.05$. The positives isolates are presented descriptively.

RESULTS

STEC subtype frequencies in bovine and ovine feces are given in Table 2. In cattle, the *Stx1a + Stx1c* subtypes (63.16%) was found to be predominant over the *Stx1a* subtype single (36.84%). *Stx1c* subtype single and the *Stx1a + Stx1d* and *Stx1c + Stx1d* were not found. Among the *Stx2* profile, the *Stx2a + Stx2c* subtype (57.57%) predominated, followed by *Stx2a + Stx2c + Stx2d* subtype (15.15%) and the *Stx2c* profile (15.15%). The subtypes *Stx2e* and *Stx2f* were not found. In sheep, the *Stx1a + Stx1c* subtypes (56.09%) was the most frequent, followed by the subtypes *Stx1c* (29.26%) and *Stx1a* (14.64%). The *Stx1d* subtype and the associations of *Stx1a + Stx1d* and *Stx1c + Stx1d* were not found. As for *Stx2*, the subtype *Stx2b* (73.91%) predominated, followed by associations of subtypes *Stx2b + Stx2g* (21.73%) and *Stx2a + Stx2c* (4.34%). The subtypes *Stx2e* and *Stx2f* were not found (Fig.1-7).

Age and antimicrobial resistance were factors ($P<0.05$) associated with isolates with greater pathogenic potential (Table 3 and 4). Adult animals were eight times more likely to have STEC with greater pathogenic potential, that is, to have *Stx2* subtypes or *Stx1 + Stx2*, compared to young animals. STEC strains with the greatest pathogenic potential were three times more likely to be MDR as compared to those with the least pathogenic potential.

Only 3.4% (2/59) of bovine strains were sensitive to all tested antimicrobials, whereas 96.6% (57/59) were resistant to at least one class of antibiotic. In sheep, 10.6% (5/47) were sensitive to all, and 89.4% (42/47) were resistant to at least one drug. In cattle 67.8% of all isolates examined were MDR, while 59.6% of sheep isolates were MDR, there was no significant difference between the proportion of isolates that were MDR when cattle and sheep were compared ($P>0.05$). The frequency of resistant strains is shown in Table 5. The strains showed greater resistance to nitrofurantoin, followed by cefotaxime and imipenem. Besides, it is important to note that the isolates of both animal species showed resistance to different antibiotics of the 1st, 2nd, 3rd, and 4th generation of cephalosporins: cefazolin, cefoxitin, cefotaxime, and cefepime, respectively.

DISCUSSION

The subtypes *Stx1a* and *Stx1c* individually or simultaneously in the same cell were found in samples of cattle and sheep. Although they bind to the same receptor, *Stx2a* is more toxic in mice, while *Stx1a* is more cytotoxic in cell culture (Russo et al. 2014). *Stx1a* is ten times more cytotoxic to Vero cells than *Stx2a*; however, the reverse is seen in mice: *Stx1a* is 100 to 400 times less lethal than *Stx2a*, even though the toxins exhibit equivalent enzymatic activities (Melton-Celsa 2014, Cherubin et al. 2019, Petro et al. 2019). According to Scheutz et al. (2012), the subtypes *Stx1a* and *Stx2a* are the most frequently associated with HUS in humans.

Table 2. *Stx1* and *Stx2* subtypes of STEC isolated from cattle and sheep feces

Animal	Subtypes	% (NP/N)
Cattle (*Stx1* = 38/*Stx2* = 33)	*Stx1a*	36.84 (14/38)
	Stx1a, Stx1c	63.16 (24/38)
	Stx2a and Stx2c	57.57 (19/33)
	Stx2a, Stx2c, and Stx2d	15.15 (5/33)
	Stx2b, Stx2c, and Stx2d	3.03 (1/33)
	Stx2c, and Stx2d	9.09 (3/33)
	Stx2c	15.15 (5/33)
Sheep (*Stx1* = 41/*Stx2* = 23)	*Stx1a*	14.64 (6/41)
	Stx1c	29.26 (12/41)
	Stx1a, and Stx1c	56.09 (23/41)
	Stx2b	73.91 (17/23)
	Stx2a and Stx2c	4.34 (1/23)
	Stx2b and Stx2g	21.73 (5/23)

N = Total number of *Stx1* or *Stx2* isolates, NP = number of positives isolates for *Stx1* or *Stx2* subtypes.

Table 3. Description of the analyzed samples of cattle and sheep feces

STEC	Cattle (59)[a]	Sheep (47)[a]
Age	16 adult/43 young	21 adult/26 young
Sex	41 female/18 male	25 female/22 male
Stx1	25[b]	25[b]
Stx2	20[c]	3[c]
Stx1 and *Stx2*	14[d]	19[d]

[a] STEC used for cattle and sheep, [b,c,d] STEC strains that have the genes *stx1*, *stx2* or both, respectively.

Table 4. Logistic regression coefficients and the odds ratios for the factors associated with the pathogenic potential in cattle and sheep

Factors	Coefficients	*P*-value	Odds ratio (CI 95%)
Age[a]	2.1139	0.0002	8.28 (2.72 - 25.17)
Sex[b]	0.3916	0.3982	1.47 (0.60 - 3.67)
Species[c]	-0.7193	0.1247	0.48 (0.19 - 1.22)
Resistance[d]	1.1585	0.0282	3.18 (1.13 - 8.96)
Intercept	-1.3319	-	-

CI = Confidence interval; Dependent variable: pathogenic potential (minor = 0, major = 1); [a] age: young = 0, adult = 1; [b] sex: male = 0, female = 1; [c] species: bovine = 0, sheep = 1; [d] resistance: NMDR = 0, MDR = 1.

Although the *Stx1a* subtype was detected in both the animal species, the association of *Stx1a* + *Stx1c* subtypes was the most frequent. Similar results were reported by Alonso et al. (2017). It is noteworthy that the simultaneity of subtypes is often found. In the study conducted by Taghadosi et al. (2018), two isolates from calves harbored five *Stx* subtypes. According to these authors, strains with a combination of *Stx1+ Stx2* genes are more cytotoxic. In the present study, 23.7% (14/59) and 40.4% (19/47) of the strains obtained from cattle and sheep, showed subtypes *Stx1* and *Stx2* simultaneously, being considered to have the greatest pathogenic potential.

The subtypes *Stx1c* and *Stx1d* are rarely associated with disease in humans, and when present in STEC, they are associated with mild disease. The low number of positive isolates for Stx1c and

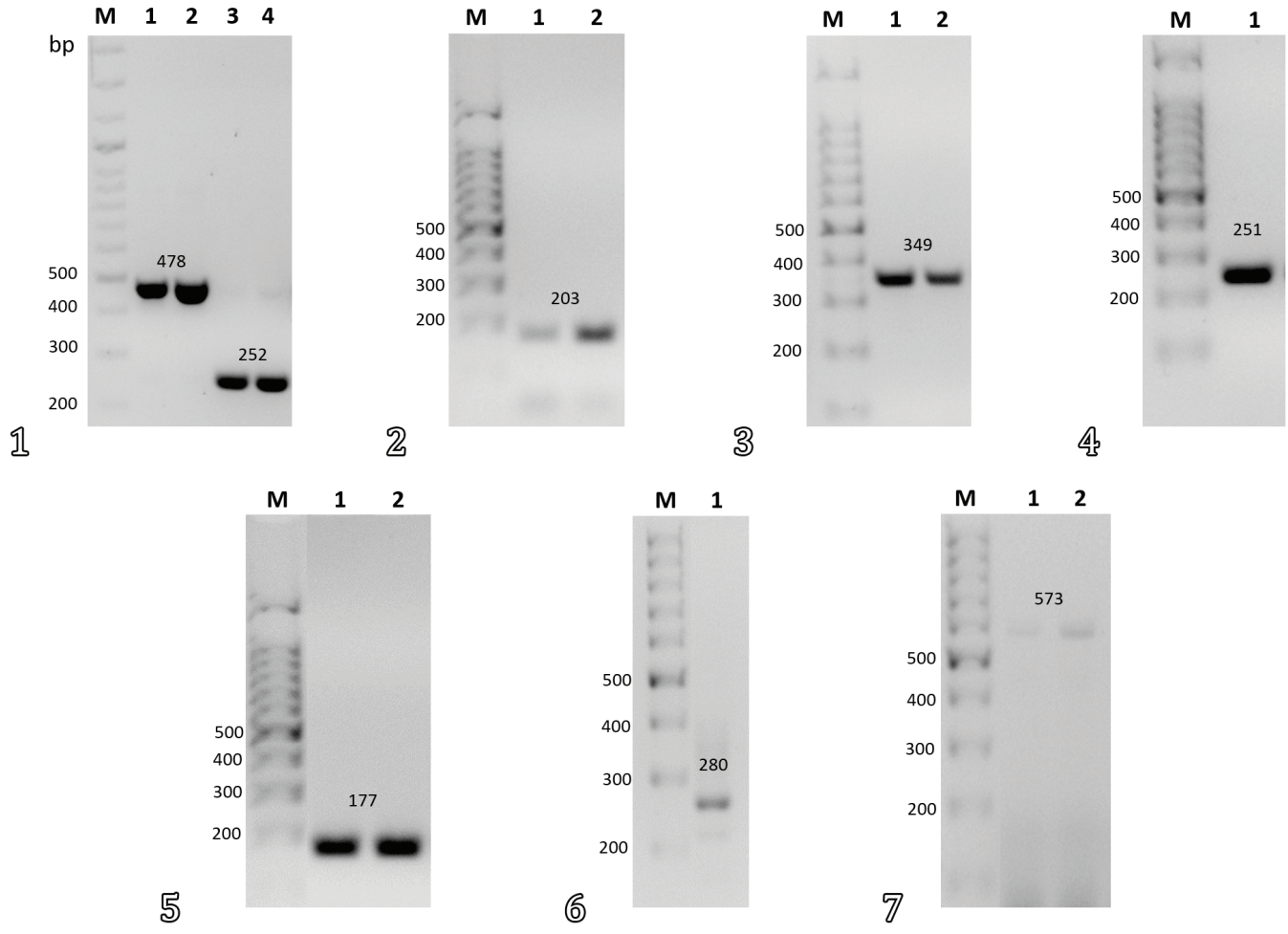

Fig.1-7. PCR products of the *Stx1* and *Stx2* subtypes genes in STEC isolates. (**1**) *stx1a* (478 pb) and *stx1c* (252 bp), (**2**) *stx1d* (203 bp), (**3**) *stx2a* (349 bp), (**4**) *stx2b* (251 bp), (**5**) *stx2c* (177 bp), (**6**) *stx2d* (280 bp) and (**7**) *stx2g* (573 bp).

Table 5. Antimicrobial resistance profile of Shiga-toxin *Escherichia coli* (STEC)

	Antimicrobial resistance profile of STEC (%)					
	Cattle (n = 59)			Sheep (n = 47)		
	Resistant	Sensitive	Intermediate	Resistant	Sensitive	Intermediate
Ampicillin	22	45.8	32.2	6.4	42.5	51.1
Cefazolin	86.4	0	13.6	74.5	0	25.5
Cefoxitin	6.8	81.3	11.9	8.5	78.7	12.8
Cefotaxime	49.1	13.6	37.3	46.8	14.9	38.3
Cefepime	18.6	8.5	72.9*	2.1	21.3	76.6*
Imipenem	40.7	23.7	35.6	10.6	55.3	34
Ciprofloxacin	6.8	62.7	30.5	6.4	68.1	25.5
Sulfazotrim	8.5	91.5	0	0	95.7	4.3
Streptomycin	27.1	11.9	61	25.5	12.8	61.7
Nitrofurantoin	57.6	15.3	27.1	53.2	21.3	25.5

* Dose-response.

Stx1d are commonly observed in other studies as well (Feng & Reddy 2013, Jajarmi et al. 2017, 2018). Studies report that the subtypes *Stx1a*, *Stx1c,* and *Stx1d* have a low association with cases of HUS (Melton-Celsa 2014, Skinner et al. 2014).

In this study, the number of isolates positives for *Stx1c* gene in sheep was found to be 29.26% (12/41) and 56.9% associated with *Stx1a*, unlike the bovine samples, where it was as only found in association with *Stx1a*, with 63.16% (24/38). According to Feng & Reddy (2013), *Stx1c* is generally associated with mild diarrhea and asymptomatic infections. It is the most common subtype among STEC strains isolated from sheep and their meat, which might justify the difference in its presence in bovine strains and sheep researched here. In other studies, with goats and calves, the subtype *Stx1c* was also reported to be most prevalent (Jajarmi et al. 2018, Taghadosi et al. 2018).

The subtype *Stx2a + Stx2c* was predominant in cattle, found in 63.16% (24/38) of the strains, while in sheep in 4.34% (1/23) of the strains. Various virulence factors were found to be associated with different subtypes of *Stx2* in the study conducted by Franz et al. (2015). The *Stx2a* was positively associated with additional virulence factors, including *eae*, unlike *Stx2b, Stx2d, Stx2*e, and *Stx2 g*, which showed a negative association with these factors. According to the same authors, the subtype *Stx2f* is generally associated with milder disease, due to the general absence of *ehxA* and *terB* genes, both of which show a significant association with HUS.

The higher prevalence of *Stx2a* is a health concern for the local population. Epidemiological data on human diseases indicate a stronger association of *Stx2a* strain with serious diseases, as compared to *Stx1a* alone. Moreover, intestine, epithelial, and endothelial cells are more sensitive to this subtype (Russo et al. 2014, Cherubin et al. 2019, Petro et al. 2019). Renal microvascular endothelial cells obtained from human glomeruli are about a thousand times more sensitive to *Stx2a* than to *Stx1*a (Melton-Celsa 2014).

In the present study, 15.15% (5/33) of STEC isolated from bovine feces had *Stx2a, Stx2c,* and *Stx2d* simultaneously, a fact of major concern, since STEC producing these combinations are often related to HUS and hemorrhagic colitis in humans (Melton-Celsa 2014, Skinner et al. 2014).

In sheep, *Stx2b* was present in 73.91% (17/23) of the strains. However, 21.73% also harbored the *stx2g* subtype. Although *Stx2b* and *Stx2d* have reduced cytotoxicity to Vero cells, and *Stx2c* toxin is more actively produced in these cells, *Stx2d* is just as toxic as *Stx2a* when injected into animals. The subtype *Stx2b* is associated with mild disease, and the subtypes *Stx2e, Stx2f*, and *Stx2 g* are closely associated with STEC infection in animals (Melton-Celsa 2014).

According to Franz et al. (2015), the *Stx2b* and *Stx2d* genes showed higher frequencies than the other genes. Our result was similar for the subtypes found in strains isolated from sheep feces. Although none of the strains had *Stx2d,* 73.91% (17/23) had the *Stx2b* gene. Martins et al. (2015), also found that the subtype *Stx2b* was the most common among sheep in Paraná, Brazil. Sheep farming is a very important animal activity in Brazil, and consequently, the risk of exposure to zoonotic pathogens is significant.

We meet higher frequency of MDR isolates, 67.8% in cattle, and 59.6% in sheep. Recent research shows high MDR-STEC rates, ranging from 66.4% (Gentle et al. 2020) to 45.45% (Yang et al. 2020). It is a known fact that these resistant bacteria can be easily transmitted from animals to humans via direct contact (Liu et al. 2016).

As per our results, 49.1% and 18.6% of the STEC strains obtained from cattle were resistant to cefotaxime and cefepime (third and fourth generation cephalosporins, respectively). In the isolates obtained from sheep, resistance to cefotaxime was not much different: 46.8%. Likewise, almost 86.4% of bovine strains, and 74.5% of sheep strains, were resistant to cefazolin (first-generation cephalosporin). Probably, the exacerbated use of third and fourth-generation cephalosporins in animals is related to the selection of resistant bacteria in them (Yu et al. 2016, Yang et al. 2020).

Among the isolates analyzed, antibiotic resistance was observed for cefazolin, cefotaxime, nitrofurantoin, and imipenem. 57.6% and 53.2% of cattle and sheep STEC were resistant to nitrofurantoin. It is noteworthy that these molecules are routinely used in human medicine mainly nitrofurantoin and imipenem, important for the treatment of urinary tract infections and complicated infections. Therefore, the "Ministério da Agricultura, Pecuária e Abastecimento" (MAPA), Brazil, have banned the use of some antimicrobials in animal production following global trends. These data demonstrate the emergence of resistant isolates important for the human population among the food-producing animal population.

Resistance to nitrofurantoin is attributed to mutations in the genes that encode nitroreductase (*nfsA* and *nfsB*) and also by plasmids that encode OqxAB efflux pumps. Studies have reported the emergence of nitrofurantoin-resistant *Escherichia coli* isolated from swine and poultry (Sørensen et al. 2003, Chen et al. 2012, Ho et al. 2016) due to the use of OLA, carbadox, mequindox, and quinocetone antimicrobials, substrates of OqxAB. According to Chen et al. (2012) and Ho et al. (2016), these isolates are not commonly found in *E. coli* isolated from cattle. Therefore, the data from the present study point to the need for further research to understand the mechanisms of nitrofurantoin resistance in STEC isolated from cattle in Brazil.

According to Rabello et al. (2020), the six main classes of antimicrobials used in the clinic for cattle and sheep are penicillins, cephalosporins, macrolides, aminoglycosides, tetracyclines, and quinolones. And among those used in animal production are of the classes: beta-lactams, tetracyclines, macrolides, aminoglycosides, and sulfonamides.

It was also found that adult animals were eight times more likely to have STEC with greater pathogenic potential than young animals. The longer exposure time of adults and longer treatment time with antibiotics might justify this fact. This is a cause of concern because human exposure to adult animals and their products is usually greater as compared to young animals. The activities such as milking and slaughtering bring humans not only in direct contact with these reservoir animals, but also with products like contaminated milk, cheese, and meat. Also, it has been observed that pathogenic STEC strains are three times more multidrug-resistant. Therefore, in addition to being highly virulent strains for humans, they can be considered difficult to treat and eliminate, due to their high resistance to antimicrobials.

The results obtained in the present study, concerning the high resistance of STEC to antimicrobials in animals from several rural properties, may indicate indiscriminate

use of antimicrobials in animals. According to Ibrahim et al. (2016), this contributes to the selection of strains that have antibiotic resistance genes and horizontal gene transfer between different species of bacteria.

CONCLUSIONS

Cattle and sheep, especially adults, are Shiga toxin-producing *Escherichia coli* (STEC) reservoirs with variants of high pathogenic potential and with a high rate of multidrug resistance to antimicrobials.

The antibiogram showed variations in the resistance profile of STEC, but the occurrence of multidrug-resistant STEC was consistent in cattle and sheep. The STEC of high pathogenic potential isolated from these animals had three times more chances of being multidrug-resistant to antimicrobials.

It is necessary to continue monitoring the susceptibility profile and evolution of resistance in microorganisms found in these animals, since they are considered sources of direct and indirect infection for humans.

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