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Detection of extended spectrum beta-lactamases and resistance in members of the Enterobacteriaceae family isolated from healthy sheep and dogs in Umuarama, Paraná, Brazil

Detecção de beta-lactamases de espectro estendido e resistência as quinolonas mediada por plasmídeos em membros da família Enterobacteriaceae isolados de ovinos e cães sadios de propriedades rurais da região de Umuarama, Paraná, Brasil

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Abstract

Bacterial resistance is a primary public health concern worldwide. Within this context, pets and breeding animals act as reservoirs for multidrug-resistant bacteria (MR), such as those producing extended spectrum beta-lactamases (ESBL) and those presenting plasmid-mediated quinolone resistance (PMQR). The aim of this study was to detect the presence of ESBL and PMQR in members of the *Enterobacteriaceae* family, isolated from healthy sheep and dogs from non-intense farming rural properties in the Umuarama region of Paraná, Brazil. A total of 81 oral and rectal swabs from dogs and sheep from 11 small rural properties were analyzed. These swabs were inoculated in tubes containing brain heart infusion broth (BHI), and the resulting cultures were inoculated on MacConkey agar (MAC) supplemented with 10 µg/mL cefotaxime for the selection of ESBL producers. The cells were also plated on MAC supplemented with 50 µg/mL nalidixic acid for selecting quinolone-resistant enterobacteria. The bacterial isolates were subjected to biochemical identification tests, antibiograms, double-disk synergic tests, and polymerase chain reaction analysis for resistance-inducing genes (bla_{ESBL}, *qnr*, and genes encoding efflux pump and acetylases). Four (5.00%) bacterial isolates (3 *Escherichia coli* and 1 *Morganella morganii*) resistant to cephalosporins and/or quinolones were identified; of these, three (75%) isolates were from sheep and one (25%) from a dog. These findings indicate the presence of MR bacteria in the normal microbiota of the animals studied. Animals colonized with such bacteria can

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contribute to the dissemination of antimicrobial resistance to other animals, environment, and/or human beings and can harbor endogenous infections in unfavorable conditions, which have poor prognosis due to the limited therapeutic options.
Key words: Antibiotics. Dogs. Drug resistance. *Enterobacteriaceae.* ESBL. *Escherichia coli*. Quinolones. *Morganella morganii.* Sheep.

Resumo

Resistência bacteriana é considerado o maior problema de saúde pública mundial da atualidade, sendo os relatos de infecções e surtos causados por bactérias multirresistentes cada vez mais frequentes na clínica veterinária e humana. Neste contexto, animais de companhia e criação podem atuar como reservatório de bactérias multirresistentes, como as produtoras de beta-lactamases de espectro estendido (ESBL) e as que apresentam resistência as quinolonas mediada por plasmídeos (PMQR). O objetivo deste trabalho foi detectar beta-lactamases de espectro estendido e resistência as quinolonas mediada por plasmídeos em membros da família Enterobacteriaceae isolados de ovinos e cães de propriedades rurais não tecnificadas da região de Umuarama, Paraná, Brasil. Foram analisados 81 *swabs* de cães e ovinos provenientes de 11 pequenas propriedades rurais da região de Umuarama (PR). Os *swabs* foram inoculados em caldo *Brain Heart Infusion* (BHI) e o crescimento obtivo foi em seguida semeado em placas de Petri contendo ágar MacConkey (MC) acrescido de cefotaxima 10 µg/mL para seleção de bactérias gram-negativas produtoras de ESBL; e placas contendo MC acrescido de ácido nalidíxico 50 g/mL para seleção de bactérias gram-negativas resistentes as quinolonas. Os isolados bacterianos obtidos foram submetidos a testes de antibiograma pelo método de disco-difusão em ágar, teste sinérgico do duplo-disco e reação em cadeia da polimerase (PCR) para genes que conferem resistência do tipo ESBL e para quinolonas. Dos 81 swabs coletados foi possível detectar quatro (4,97%) isolados bacterianos (3*Escherichia coli* e 1 *Morganellamorganii*) resistentes a cefalosporinas e/ou quinolonas. Destes isolados, três (75%) eram de ovinos e um (25%) de cão. Os resultados encontrados indicam a presença de cepas multirresistentes na microbiota normal dos animais estudados. Nesta condição, os animais colonizados podem contribuir para disseminação dos agentes bacterianos para outros animais, ambiente e/ou homem, ou em uma situação desfavorável o hospedeiro pode adquirir uma infecção endógena, com prognóstico desfavorável decorrente da falha terapêutica mediada pela expressão de genes de resistência para antibacterianos considerados de última escolha terapêutica.
Palavras-chaves: Antimicrobianos. Cães. ESBL. *Enterobacteriaceae*. *Escherichia coli. Morganella morganii.* Ovinos. Quinolonas. Resistência bacteriana.

Introduction

Antimicrobial resistance is considered the primary public health issue in the current global scenario. Infections and outbreaks caused by multidrug-resistant bacteria are frequent in both human and veterinary medicine, and in some cases, the isolated microorganisms are resistant to all available antimicrobial drugs (SOUZA, 2010; JONG et al., 2011; TEO et al., 2012; WHO, 2014).

The emergence and selection of multidrug-resistant strains is related to the inappropriate use of antimicrobial drugs during treatment, as well as their use as growth promoters and for prophylaxis in veterinary medicine. In both cases, the antibiotic dosage is lower than that in therapeutic use, easing the appearance of mutations and/or acquisition of resistance genes due to selective pressure (CARATTOLI, 2008; SOUZA et al., 2010; ISHII et al., 2011).

Studies in different countries have reported the presence of cephalosporin and quinolone residues in animal-derived products such as meat, milk, and eggs. This situation increases the risk of emergence or selection of multidrug-resistant strains, such as those producing extended spectrum beta-

lactamases (ESBL) and those resistant to quinolones (VRAGOVIĆ et al., 2011; WHO, 2011). By definition, ESBL-producing strains are resistant to penicillins, cephalosporins, and monobactams, but are sensitive to clavulanate (PEIRANO et al., 2011). These enzymes hydrolyze the beta-lactamic ring on the antibiotic, resulting in its deactivation. Currently, there are more than 370 known ESBL variants, most of which are encoded by plasmidial genes. The most frequently identified ESBL variants are TEM, SHV, OXA, and CTX-M (MINARINI et al., 2007; PEIRANO et al., 2011; LAGO et al., 2010).

Quinolones represent the antibiotic category used in the feeding of animals, thereby enabling the selection of resistant strains and consequently allowing these production animals to act as potential reservoirs and transmitters of multidrug-resistant bacteria to other animals, the environment and also to humans (WHO, 2011).

Resistance to quinolones can be caused by mutations in the genes encoding topoisomerase and DNA gyrase or by the presence of resistance plasmids. The latter mechanism is known as plasmid-mediated quinolone resistance (PMQR) (STRAHILEVITZ et al., 2009; KARAH et al., 2010; RODRÍGUEZ-MARTÍNEZ et al., 2011). Known PMQR mechanisms include: (1) the protection of gyrase and topoisomerases mediated by *qnr* genes (ROBICSEK et al., 2006a; CATTOIR; NORDMANN, 2009; KARAH et al., 2010; STRAHILEVITZ et al., 2009; BAE et al., 2010; RODRÍGUEZ-MARTÍNEZ et al., 2011; ZHAO et al., 2010); (2) acetylation of quinolones and fluoroquinolones mediated by the *aac(6´)-Ib-cr* gene (ROBICSEK et al., 2006b; CATTOIR; NORDMANN, 2009; JACOBY et al., 2009; KARAH et al., 2010; STRAHILEVITZ et al., 2009; RODRÍGUEZ-MARTÍNEZ et al., 2011); and (3) efflux pumps that expel quinolones and fluoroquinolones from the bacterial cell (CATTOIR; NORDMANN, 2009; MA et al., 2009; CATTOIR; NORDMANN, 2009; STRAHILEVITZ et al., 2009; BAE et al., 2010; KARAH et al., 2010; HERRERA-LEÓN et al., 2011).

In recent years, the frequency of isolating ESBL-producing and/or quinolone-resistant members of *Enterobacteriaceae* has increased because infections caused by these bacteria have limited therapeutic alternatives, causing grave concern among health authorities and institutions (ALDRED et al., 2014; CAUMO et al., 2010). Studies involving different animal species, including production animals and pets, have isolated *Enterobacteriaceae* family members producing different types of ESBLs, including TEM, SHV, and CTX (PEIRANO et al., 2011; LAGO et al., 2010).

Animals colonized by ESBL-producing and/or PMQR harboring strains can be asymptomatic carriers, and invariably contribute to dissemination of these bacterial agents to other animals and/or humans. Alternatively, such animals can harbor an endogenous infection in unfavorable conditions, which have poor prognosis due to therapeutic failures, as measured by the expression of resistant genes to antibacterial drugs considered as the last therapeutic choice. The aim of this paper was to detect ESBL-producing and/or quinolone-resistant strains among *Enterobacteriaceae* family members isolated from healthy sheep and dogs from rural properties in the Umuarama region of Paraná, Brazil.

Materials and Methods

Ethics committee

This project was submitted to the Ethics Committee in Animal Experimentation (*Comitê de Ética em Experimentação Animal* – CEPEEA) at UNIPAR and was approved under protocol number 25113/2014 on 25/Jul/2013.

Location & sampling

A total of 81 swabs were collected from dogs and sheep, in the period of February to June 2014. Of these 81 swabs, 50 were collected from the rectal cavity of mixed breed sheep in their reproductive

age (19 male and 31 female), and 31 from the oral cavity of mongrel dogs of age greater than one year (17 male and 14 female), from 11 small non-intense farming properties for sheep exploitation in the region of Umuarama, in the northeastern region in the state of Paraná, Brazil.

In the small rural properties studied, the sheep were raised for subsistence and as a source of income. Dogs were present in all these rural properties. No animal (whether sheep or dog) presented clinical signs of infectious disease upon physical examination.

Collection of samples

Rectal and oral samples were collected using swabs containing AIMES medium + activated charcoal (Copan Transystem©, Italy), which were introduced in the rectal ampoule of each sheep, compressing it with rotating moves; and in the oral cavity of dogs, with circular and rotating moves performed in the gingiva and tongue regions. All samples were preserved and sent under refrigeration to the Laboratory of Preventive Veterinary Medicine and Public Health from the Masters Program in Animal Science at the Paranaense University (UNIPAR) for subsequent analysis.

Bacterial isolation and identification

Tubes containing 3.0 mL of brain heart infusion (BHI) medium were inoculated with the collected rectal and oral swabs and incubated for 24 hours at 37 °C. Subsequently, the cultures inoculated on Petri plates containing MacConkey agar supplemented with 10 µ/mL cefotaxime (CTX) and MacConkey agar supplemented with 50 µg/mL nalidixic acid (NAL) and incubated for 24 hours at 37 °C to isolate colonies resistant to cephalosporin and quinolones. The isolated colonies were stored in BHI medium + glycerol 80% solution at –20 °C for preservation.

Biochemical identification of bacterial isolates

Biochemical identification of bacteria belonging to the *Enterobacteriaceae* family was performed using the "*Kit para Enterobactérias* (Enterobacteria kit)" (NewProv®, Paraná, Brazil), following the manufacturer's recommendations.

Phenotypic tests for antimicrobial susceptibility

To determine the antibiotic resistance profile, the disk-diffusion in agar method was used, as per recommendations of the Clinical and Laboratory Standards Institute (CLSI, 2013). The disks tested were: gentamicin (GEN), ciprofloxacin (CIP), ceftazidime (CAZ), sulphazotrim (SUT), amikacin (AMI), aztreonam (ATM), chloramphenicol (CLO), ampicillin (AMP), tobramycin (TOB), cefoxitin (CFO), ceftriaxone (CRO), cefotaxime (CTX), tetracycline (TET), amoxicillin (AMO) and amoxicillin + clavulanate (AMC), imipenem (IMP), meropenem (MER), norfloxacin (NOR), and nalidixic acid (NAL). As the bacterial samples were isolated from animals, enrofloxacin (ENO) and ceftiofur (CEF) sensitivity was also tested, since they are antimicrobial drugs exclusively for veterinarian use.

Phenotypic tests for detection of ESBL-producing strains

The double-disk synergy test was performed to detect ESBL-producing strains. Briefly, disks containing CTX, CAZ, CRO, and ATM were distributed at a 20 mm-distance from a disk containing AMC (20/10 µg). Any increase or distortion in the zone of inhibition for one of the antibiotics towards the AMC disk was considered as suggestive for ESBL production (BRUN-BUISSON et al., 1987).

Genotyping for the detection of ESBL and quinolones

The bacterial DNA was obtained by the boiling method and the ESBL genes bla_{TEM}, bla_{SHV}, and bla_{CTX-M} (including $bla_{CTX-M-2}$, $bla_{CTX-M-8}$, and $bla_{CTX-M-15}$) and quinolone-resistance genes (*qnr* and genes encoding acetylases and efflux pump) were identified by polymerase chain reaction (PCR). The primers and reaction conditions are described in Table 1.

Table 1. Initiators and annealing temperatures for the detection of bacterial resistance genes (ESBL and quinolones) used in the isolates from sheep and dog in rural properties exploiting sheep production in the region of Umuarama, Paraná, Brazil, 2014.

Type	Gene	Primers (5`-3`)	Fragment (pb)/ Temp. (ºC)	Reference
ESBL	bla_{TEM}	F- GAGTATTCAACATTTCCGTGTC R- TAATCAGTGAGGCACCTATCT	861/51	Poirel et al. (2000)
ESBL	bla_{SHV}	F- ATGCGTTATATTCGCCTGTG R- GTTAGCGTTGCCAGTGCTCG	573/53	Minarini et al. (2007)
ESBL	bla_{CTX-M}	F-CGCTTTGCGATGTGCAG R- ACCCGCATATGCTTGTG	544/56	Bonnet et al. (2000)
ESBL	$bla_{CTX-M-2}$	F- GCGACCTGGTTAACTACAATC R- CGGTAGTATTGCCCTTAAGCC	351/55	Nedjai et al. (2012)
ESBL	$bla_{CTX-M-8}$	F- CTGGAGAAAAGCAGCGGGGG R- ACCCAGGATGTGGGTAGCCC	320/55	Minarini et al. (2007)
ESBL	$bla_{CTX-M-15}$	F- CACACGTGGAATTTAGGGACT R- GCCGTCTAAGGCGATAAACA	550/56	Muzaheed et al. (2008)
Quinolonas (PMQR)	*qnrA*	F- ATTTCTCACGCCAGGATTTG R- TGCCAGGCACAGATCTTGAC	468/53	Jacoby et al. (2009)
Quinolonas (PMQR)	*qnrB*	F- CGACCTKAGCGGCACTGAAT R- GAGCAACGAYGCCTGGTAGYTG	513/53	Jacoby et al. (2009)
Quinolonas (PMQR)	*qnrS*	F- ACTGCAAGTTCATTGAACAG R- GATCTAAACCGTCGAGTTCG	431/53	Jacoby et al. (2009)
Quinolonas (Efluxo)	*qepA*	F- AACTGCTTGAGCCCGTAGAT R- GTCTACGCCATGGACCTCAC	595/65	Yum et al. (2005)
Quinolonas (Acetilase)	*aac(6`)-1b*	F- TTGCGATGCTCTATGAGTCGCTA R- CTCGAATGCCTGGCGTGTTT	459/55	Park et al. (2006)

Results and Discussion

Analysis of the 81 isolated swabs led to the identification of four (5.00%; 4/81 swabs) bacterial isolates resistant to beta-lactams and/or quinolone antibiotic drugs. Among these isolates, three (75%) were from sheep and one (25%) was from a dog.

Strains resistant to beta-lactam antibiotics alone were identified in 1.25% (1/81 swabs) of the animals studied. Resistance only to quinolones was observed in 2.50% (2/81 swabs) and resistance to beta-lactams associated with quinolone resistance was seen in 1.25% (1/81) of the animals studied.

One (3.20%; 1/31 swabs) ESBL-producing *Escherichia coli* was detected in the oral cavity swabs of dogs, and its resistance profile was confirmed by the double-disk synergic test and the detection of $bla_{CTX-M-15}$ and bla_{TEM} via PCR (Figure 1A and Table 2).

In swabs collected from sheep, two *Escherichia coli* (4.0%; 2/50 swabs) strains resistant to all quinolones tested were identified. Both bacteria were isolated from female sheep and *qnr* genes were detected in PCR analysis, confirming the profile found in the antibiogram (Table 2).

ESBL resistance was determined in one *Morganella morganii* strain (2.0%; 1/50 swabs) isolated from a male sheep, as confirmed by the presence of the $bla_{\text{CTX-M-2}}$ gene detected by PCR. Other resistance genes were also identified in the same isolate, including the genes $bla_{\text{CMY-2}}$ and *qnrA*, granting AmpC-producing ability and PMQR to this strain (Figure 1B and Table 2).

The results of all the tests performed, as well as the resistance profiles obtained through antibiograms, are depicted in Figures 1 and 2 and described in Table 2.

The current antibiotic-therapy crisis has raised the need to generate public policies on the rational use of antibacterial drugs. Unfortunately, in Brazil, such actions have been directed mainly towards the use of antibiotics in human medicine. There still remain several discrepancies in the veterinarian sector, probably due to the lack of information on the impact of using antibacterial drugs in this area, as well as due to the lack of epidemiological studies that could provide panoramic insights into bacterial resistance in the residing microbiota in pets and production animals.

Phenotypic and genotypic tools such as the antibiogram and PCR, allow the monitoring of the dissemination of resistance determinants, identifying sources and animal housing multidrug-resistant bacteria that can be transferred to human beings, the environment or other animals. The presence of gram-negative bacilli carrying ESBL genes in pets and production animals has been reported in several studies. However, these studies are limited in Brazil, especially on the oral cavity of pets, as current research has mainly focused on the analysis of feces and urine samples (MORENO et al., 2008; MA et al., 2009, 2012).

Some studies have detected high isolation frequencies of ESBL-producing *E. coli* in pets and production animals (MORENO et al., 2008; MA et al., 2009, 2012). The isolates harbored $bla_{\text{CTX-M-1}}$, $bla_{\text{CTX-M-2}}$, $bla_{\text{CTX-M-8}}$ or $bla_{\text{CTX-M-15}}$ genes and, in some cases, a single strain presented more than one type of ESBL gene, with the presence of $bla_{\text{CTX-like}}$ associated with other ESBL genes, such as bla_{TEM} (MORENO et al., 2008).

A study performed by Moreno et al. (2008) identified a 20% prevalence of ESBL-producing strains in feces and urine from pets. A few isolates presented more than one ESBL variant. This paper presents alarming data on beta-lactam antibiotic-resistant strains in pets, with the threat of dissemination of such multidrug-resistant strains via the feces of these animals into the environment they live in.

Figure 1. A) ESBL production in *Escherichia coli* (P1C4 strain) isolated from the oral cavity of a dog. The distortion in inhibition halos indicated by the arrows highlight ESBL production. B) Double-disk test for the *Morganella morganii* strain (P4O11 Strain), isolated from a male sheep, indicating the production of AmpC. The sheep and dog originated from a rural property in the region of Umuarama, Paraná, Brazil, 2014.

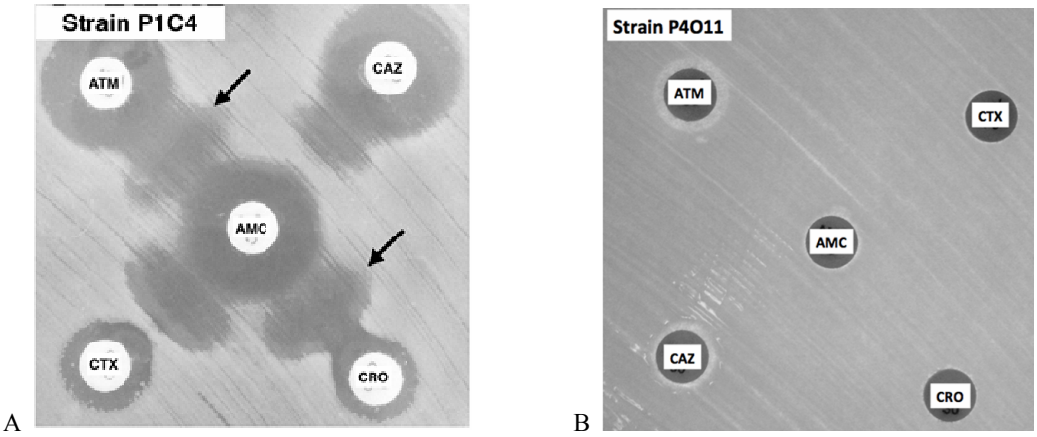

Table 2. Phenotypic and genotypic profiles of ESBL-producing isolates resistant to quinolones from sheep and dog isolated from rural properties exploiting sheep production in the region of Umuarama, Paraná, Brazil, 2014.

Isolate	Animal	Species	Sex[1]	Profile of resistance[3]																						Profile of resistance[2]	Genotypic profile
				NOR	ENO	NAL	CIP	TOB	AMI	GEN	MER	IPM	ETP	CAZ	CRO	CTX	CTF	CFO	AMP	AMO	AMC	ATM	SUT	TET	CLO		
P1C4	Dog	*E. coli*	M	S	S	S	S	S	S	S	S	S	S	R	R	R	R	R	R	R	S	R	S	S	R	ESBL	$bla_{CTX-M15}$; *bla_{TEM}*
P1O11	Ovine	*E. coli*	F	R	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R	I	PMQR	*qnrB*
P4O11	Ovine	*M. morganii*	M	R	R	R	R	S	S	S	S	S	S	R	R	R	R	R	R	R	R	R	S	S	S	ESBL + PMQR	*$bla_{CTX-M-2}$*; *bla_{CMY-2}*; *qnrA*
P6O2	Ovine	*E. coli*	F	R	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	PMQR	*qnrA*; *qnrD*

R – Resistant; S – Sensitive; I – Intermediary;
[1]M – Male; F- Female;
[2]ESBL – Extended-Spectrum Beta-Lactamases; QUI – Quinolones;
[3]NOR – norfloxacina; ENO – enrofloxacina; NAL – ácido nalidíxico; CIP – ciprofloxacina; TOB – tobramicina; AMI – amicacina; GEN – gentamicina; MER – meropenem; IPM – imipenem; ETP – ertapenem; CAZ – ceftazidima; CRO – ceftriaxona; CTF – ceftiofur; CFO – cefoxitina; AMP – ampicilina; AMO – amoxicilina; AMC – amoxicilina + clavulanato; ATM – aztreonam; SUT – sulfametazol-trimetoprima ; TET – tetraciclina; CLO – cloranfenicol

Phenotypic and genotypic data from the isolates found in the work of Moreno et al. (2008) corroborate the results of the present study, as the *E. coli* (P1C4) strain isolated from the oral cavity of dogs presented the $bla_{\text{CTX-M-15}}$ and bla_{TEM} genes. On the other hand, the low isolation frequency suggests that such a bacterium may be present only transitorily in the oral cavity of dogs.

Nevertheless, the presence of ESBL-producing *E. coli* found in a dog oral sample indicates a high risk of contamination between animals and humans, since there is frequent contact with dog saliva. Moreover, as these animals were living in rural properties, they may acquire these strains from other animals or even human beings, as described by Moura et al. (2009), who studied the crossed-transmission of enterobacteria between humans and pets.

Along with beta-lactam antibiotics, quinolones are also used for therapeutic purposes, prophylaxis or growth promotion in animal production environments (AARESTRUP, 2005). Abuse in their use has led to selection and development of quinolone-resistant bacteria, compromising the treatment of infectious diseases in these animals (AARESTRUP, 2005; GIBBS et al., 2006; MA et al., 2009; HERRERA-LEÓN et al., 2011).

Several studies have shown the emergence and dissemination of PMQR in enterobacteria isolated from pets, production and wild animals (LIU et al., 2008; HUANG et al., 2009; MA et al., 2009; POMBA et al., 2009; ASAI et al., 2010; GIBSON et al., 2010a, 2010b; FORTINI et al., 2011). These data indicate that these animals are an important reservoir for PMQR-resistance genes and they can act as possible transmission vectors for human beings, the environment and other animals (ROBICSEK et al., 2006a; GIBBS et al., 2006; HAWKEY et al., 2009).

Quinolone resistance attributed to the presence of *qnr* genes was detected in 2.50% (2/81 swabs) of sheep and dogs studied, and *E. coli* was isolated in both cases. Genes *qnrA* and *qnrD* were identified in these two strains, with one of them presenting the two subtypes.

The results indicate a low prevalence of quinolone resistance. However, the resistance profile in enterobacteria isolated from pets is similar to the profile of quinolone-resistant enterobacteria isolated from humans, indicating that animal-human-environment cross-transmission relations are possible (POMBA et al., 2009; FERREIRA et al., 2010a, 2010b; ANTUNES et al., 2011).

Quinolone-resistant genes have been identified in enterobacteria isolated from different animal species, including commensal microbiota of pets and production animals, suggesting that these animals are important reservoirs for such bacteria (NOVAIS et al., 2005; MACHADO et al., 2008).

Additionally, an association between *qnr* and ESBL production in single enterobacteria has been reported in a few studies (POMBA et al., 2009). The present study has identified a *M. morganii* strain producing ESBL, AmpC and resistance to quinolones isolated from the rectal cavity of a male sheep, with its phenotypic and genotypic profile, corroborating results from studies performed in humans, pets and production animals around the world. AmpC production was confirmed by means of the antibiogram profile and by the detection of the $bla_{\text{CMY-2}}$ gene through PCR. The presence of this gene enables this strain to be resistant to AMC, as AmpC-type beta-lactamase producing bacteria can inhibit the action of clavulanate (MACHADO et al., 2006; TOUATI et al., 2008; MINARINI et al., 2008; CRÉMET et al., 2011; PANIAGUA et al., 2010; HUANG et al., 2009; MAHROUKI et al., 2013).

Importantly, all resistance determinants found in the studied strains are coded by mobile genetic elements, easing the dissemination of these resistance genes among strains of the same species, as well as among different species within the microbiota of an animal.

Conclusion

This study suggests a possibility of direct and/or indirect transmission of resistant bacteria through human-animal contact, as well as their transmission to the environment, as the bacteria isolated from animals and human beings present the same phenotypic and genotypic profiles. The results underscore the need for further molecular studies to characterize the genes coding for the acquired resistance to quinolones in other environmental niches, detect the emergence of new resistance genes and monitor their dissemination among different bacteria and environmental niches. Only then will it be possible to design and implement efficient measures to control the dissemination of multi-resistant bacteria (including animal to human transmission) and minimize the risk for human health.

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