http://dx.doi.org/10.1590/1678-4162-12805

Arq. Bras. Med. Vet. Zootec., v.75, n.3, p.525-530, 2023

Communication

[*Comunicação*]

Resistance profile of bacterial isolates from different llama microbiotas (*Lama glama* Linnaeus 1758)

[*Perfil de resistência de isolados bacterianos de diferentes microbiotas de lhamas* (Lama glama *Linnaeus 1758*)]

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The New World camelids are ungulate animals, members of the Camelidae family, among these members are the llamas (*Lama glama* Linnaeus 1758), considered very agile animals that inhabit arid and semi-arid places, temperate pastures, and deserts, being found, mainly, in the Andes, and feed basically on grasses and foliage of some shrubs (Yacobaccio and Vilá, 2016; Vilá and Arzamendia, 2020; Yacobaccio, 2021).

Llamas have an important role in society, especially regarding the growing creation of these animals for sale, hobby, and even with the objective of turning them into pets due to the fact that they are considered docile and easy-to-handle animals (Stahl, 2008; Schauer *et al*., 2018).

The approach of human beings with these camelids raises questions about the One Health that involves this animal species, the human, and the environment in which they live. There are few works aimed at researching the microorganisms that are part of the microbiota of these animals (Schauer *et al*., 2018; Stahl, 2008). In view of this situation, the aim of this manuscript was to identify the profile of the bacterial microbiota as well as the respective bacterial resistances of domesticated llamas (*Lama glama*) isolates.

Four llamas (*Lama glama*) were admitted to the Veterinary Hospital of the Universidade Paranaense (UNIPAR), two males and two females, one of them less than 24 years old, for routine examinations. The animals were hospitalized so that their health could be checked. As part of the history of these animals, it was declared by the owner that they lived with farm animals and that there were no previous illnesses.

During the dental procedure with retention, swabs were collected from the mouth, nose, ear, skin, rectum and foreskin in males, and vagina in the case of the female, as part of a battery of tests requested by the responsible veterinarian.

The collected swabs were inserted into tubes containing Brain Heart Infusion (BHI). After 24 hours incubated at 37°C, each sample was seeded in Mannitol Salt agar and MacConkey agar and incubated at 37°C for 24 hours for isolation of Gram positive bacteria and negative (Quinn *et al*., 2005). Then, each colony was submitted to analysis of macroscopic and microscopic characteristics and biochemical tests, allowing the identification of these microorganisms.

Gram-positive isolates were classified using catalase and coagulase tests, allowing them to be classified into coagulase positive *Staphylococcus* (CoPS) and coagulase negative *Staphylococcus* (CoNS) (Quinn *et al*., 2005).

Gram-negative isolates were identified using the "Enterobacteria Kit" (NewProv®, Paraná, Brazil), according to the manufacturer's recommendations.

Antimicrobial susceptibility testing was performed according to the Clinical and Laboratory Standards Institute (Performance…, 2018).

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Submitted: June 13, 2022. Accepted: January 10, 2023.

CoPS and CoNS isolates were subjected to disk diffusion test against amikacin (30µg), ampicillin (10µg), cephalotin (30µg), cefoxitin (30µg), clindamycin (2µg), enrofloxacin (5µg), gentamicin (10 µg), norfloxacin (10µg), oxacillin (1µg), penicillin G (10U), sulfazotrim (25µg).

Each Enterobacterial isolate was submitted to disk diffusion test where they were tested against antibiotics; nalidixic acid (30µg), amikacin (30µg), amoxicillin (10µg), amoxicillin + clavulanic acid (30µg), ampicillin (10µg), cefepime (30µg), cefotaxime (30µg), ceftazidime (30µg), ceftiofur (30µg), ceftriaxone (30µg), ciprofloxacin (05µg), chloramphenicol (30µg), enrofloxacin (05µg), ertapenem (10µg), gentamicin (10µg), imipenem (10µg), meropenem (10µg), sulfazotrim (25µg), tetracycline (30µg) and tobramycin (10µg).

The phenotypic test for detection of ESBL-producing enterobacteria was performed by the double-disc synergistic test with cefotaxime (30 µg), ceftazidime (30µg), ceftriaxone (30µg) and aztreonam (30µg). The discs were distributed at 20 mm from a disc containing amoxicillin + clavulanic acid (20/10µg). Any increase or distortion of the inhibition zone of one of the antibiotics towards the amoxicillin + clavulanate disc was considered suggestive of ESBL production (Brun-Buisson *et al*., 1987).

CoPS isolates were subjected to DNA extraction with the aid of the PurelinkGenomic DNA Kit (Invitrogen, Carlsbad, California, USA) following the manufacturer's instructions, and then the reactions were performed using the primer Sa442-1 (5'-AAT CTT TGT CGG TAC ACGATA TTC TTC ACG-3' and primer Sa442-2 (5'-CGT AAT GAGATT TCA GTA GAT AAT ACA ACA-3') following the methodology of Martineau *et al*. (1998) The amplification product was visualized by electrophoresis in a 2% agarose gel stained with GelRed (Uniscience, Osasco, São Paulo, BR) using a 100bp molecular marker, the amplified products were visualized as a single band of 241bp.

DNA from *Staphylococcus* spp. classified as resistant to oxacillin were extracted and PCR reactions were performed using the primers mecA1 (AAAATCGATGGTAAAGGTTGG) and mecA2 (AGTTCTGCAGTACCGGATTTG) following the methodology of Murakami *et al*. (1991). The amplification of the products was visualized by electrophoresis on a 2% agarose gel stained with GelRed (Uniscience, Osasco, São Paulo, BR) using a 100bp molecular marker and these products were visualized as a single 533bp band.

Of the 24 samples collected, five (20.83%) were isolated *Staphylococcus* spp. coagulase-positive, and 19 (79.17%) *Staphylococcus* spp. coagulase-negative and in relation to the enterobacteria isolated in these samples, 19 (79.17%) were identified as *Escherichia coli*¸ two (8.35%) as *Pantoea agglomerans*, one (4.16%) as *Koserella trabulsii*, *Enterobacter aerogenes* and *Klebsiella Pneumoniae* each (Table 1).

Table 1. Classification and identification of Gram-negative and Gram-positive isolates from swabs from llamas (*Lama glama* Linnaeus 1758**)** treated at a University Veterinary Hospital in Umuarama, Paraná, Brazil, 2019

Llamas	Swabs	Identification		Llamas	Swabs	Identification	
		MSA	MC			MSA	MC
Adult Male	Ear swab	CoNS	*E. coli*	Adult Female	Ear swab	CoNS	*E.coli*
	Skin swab	CoNS	*E. coli*		Skin swab	CoPS	*E. coli*
	Nasal swab	CoNS	*E. coli*		Nasal swab	CoPS	*E. coli*
	Oral swab	CoNS	*E. coli*		Oral swab	*S. aureus*	*E. coli*
	Rectal swab	CoNS	*E. coli*		Rectal swab	CoNS	*E. coli*
	Preputial swab	CoPS	*E. coli*		Vaginal swab	CoPS	*E. coli*
Cria Male	Ear swab	CoNS	*E. coli*	Cria Female	Ear swab	CoNS	*Pantoea (Enterobacter) agglomerans*
	Skin swab	CoNS	*E. coli*		Skin swab	CoNS	*Pantoea (Enterobacter) agglomerans*
	Nasal swab	CoNS	*E. coli*		Nasal swab	CoNS	*Koserella trabulsii*
	Oral swab	CoNS	*E. coli*		Oral swab	CoNS	*E. coli*
	Rectal swab	CoNS	*E. coli*		Rectal swab	CoNS	*E. aerogenes*
	Preputial swab	CoNS	*E. coli*		Vaginal swab	CoNS	*Klebsiella Pneumoniae*

Legend: MSA = Mannitol Salt Agar; MC = MacConkey agar; CoPS = Coagulase-positive *Staphylococcus*; CoNS = coagulase-negative *Staphylococcus*.

The isolates that were classified as CoPS were submitted to PCR to identify *Staphylococcus aureus*, with only one (20%) being isolated from the oral swab of an adult female positive for this microorganism.

In all isolates in the present study, the presence of CoPS or CoNS was identified, such results corroborate the findings of Tibary *et al*. (2006), Jarvinen and Kinyon (2010), Schauer *et al*. (2018, 2021), where CoNS and CoPS were isolated from different microbiota of domesticated llamas, belonging to herds and/or described as companion animals by their owners.

Researchers in this area, however, already know the presence of CoNS and CoPS in a wide range of locations and microbiota the research of these microorganisms in different microbiota of animals and humans has become of great importance, especially for the evaluation of the profiles of resistance carried by these.

The transfer and sharing of microorganisms between humans and animals have been described in different studies, some of which are even considered zoonotic agents. For this reason, the research and evaluation of microorganisms present in animals increasingly closer to man becomes a major concern for One Health, especially when considering the importance of these isolates in relation to the bacterial resistance they carry (Argudín *et al*., 2017; Mourabit *et al*., 2020).

Escherichia coli was identified in 79.17% of samples collected from different llama microbiota, the presence of this microorganism in these animals has already been described as related to reproductive diseases, as well as *Klebsiella Pneumoniae* and *Enterobacter aerogenes*, found each in one of the samples (4.16%) of the present work, being cited as common pathogens in uterine infections in this animal species (Tibary *et al*., 2006). However, this statement does not corroborate the findings of this study, where the animals that had their samples taken were considered healthy at the time of collection.

In the present work, two samples (8.35%) of *Pantoea agglomerans* were also isolated, which is an endophytic bacterium, which can be found in all plant species, and is not present in humans and animals (Dutkiewicz *et al*., 2016), unlike *Koserella trabulsii* (*Yokenella regensburgei*), which is commonly found in humans, in addition to reptiles and in water (Milori *et al*., 2017).

The isolation of these bacteria in healthy animals can be considered as a transient microbiota, acquired from the environment these animals frequented, especially when considering that the identification of the isolates shows the presence of microorganisms widely found in the environment, and which may, or may not, be pathogenic bacteria (Jang *et al*., 2017).

Among *Staphylococcus* spp. isolates, 19 (79.17%) were resistant to sulfazotrim, followed by penicillin with 11 (45.83%), ampicillin with five (20.83%), clindamycin and oxacillin with four (16, 67%) each and amikacin, cephalotin, and enrofloxacin with one (4.17%) each, with no resistance against cefoxitin, gentamicin, norfloxacin, and vancomycin.

Isolation of *Staphylococcus* spp. antimicrobial resistant in these camelids has already been described by Schauer *et al*. (2018, 2021), who in their works reported the research of *Staphylococcus* spp. Methicillin resistant in new world camelids, where they found isolates resistant to beta-lactams, a result that corroborates the present study, where resistance to this class was found in 50% (12) of the isolates.

In the disk diffusion test for enterobacteria, 14 (58.3%) of these were resistant to amoxicillin + clavulanic acid and cefotaxime, 12 (50%) to ceftazidime and ceftriaxone, eight (33.3%), seven (29, 2%) and six (25%) were, respectively, resistant to amoxicillin, ampicillin, and sulfazotrim, 4 (16.7%) to nalidixic acid, enrofloxacin and tetracycline, three (12.5%) to ciprofloxacin, two (8 .33%) to ertapenem and one (4.17%) was resistant to cefepime, ceftiofur, gentamicin, meropenem and tobramycin. On the other hand, none of the isolates was classified as resistant to amikacin, chloramphenicol and imipenem. Even among the Gram-negative isolates, there was no phenotypic expression of ESBL isolates.

Due to this situation, studies on the microorganisms present in this animal species, as well as their antibacterial resistance profiles, are

essential for One Health, especially when carrying and sharing these samples both with the environment, with man and with other animal species is taken into account (Loncaric *et al*., 2016; Argudín *et al*., 2017; Shen *et al*., 2020).

An important factor that may be involved in the resistance profile of these isolates, *Quorum sensing* is a communication mechanism between bacteria, which allows them to regulate gene and phenotypic expression as a kind of response to fluctuations in cell population density. The likely presence of a sensor may elucidate the high presence of bacterial resistance in isolates that are poorly exposed to antimicrobials (Hawver *et al*., 2016; Moreno-Gámez *et al.,* 2017; Mion *et al.*, 2021).

Among the oxacillin resistant isolates, the presence of the *mec*A gene was confirmed in only one (0.19%) isolate from the bacterial microbiota of the adult male skin.

As in the present work, Schauer *et al.* (2021) detected the presence of the *mec*A gene in CoNS samples, the detection of this gene in CoNS samples is still poorly described in the literature, which may be because such microorganisms were considered of clinical importance only recently, however, these findings show the spread of resistance genes among unconventional animal species (Schauer *et al*., 2021).

The present work exposed the presence of microorganisms resistant to antibiotics in llamas that had not previously had any contact with these drugs, in addition to the presence of the *mec*A gene in one of the animals. For this reason, more research involving new world camelids should be carried out, especially when considering their growing proximity to man and other domestic and domiciled animals.

Knowledge of the bacterial microbiota of different animal species has become increasingly important, especially when considering their resistance profile and the possible genes involved in this profile. Such relevance is due to the possibility of these microorganisms being shared between animals, humans and even the environment.

Keywords: Llamas, *mec*A gene, one health, resistance

RESUMO

Este trabalho objetivou o isolamento e a identificação das bactérias de diferentes microbiotas de lhamas. Para tanto, testes de disco difusão e diagnósticos moleculares para pesquisa de genes de resistência foram realizados. Foram isolados cinco Staphylococcus *spp. coagulase positiva e 19* Staphylococcus *spp. coagulase negativa, 19* Escherichia coli¸ *duas* Pantoea agglomerans, *uma* Koserella trabulsii, *uma* Enterobacter aerogenes *e uma* Klebsiella Pneumoniae. *Entre os isolados de* Staphylococcus *spp., 79,17% foram resistentes ao sulfazotrim, 45,83% resistentes à penicilina e 20,83% à ampicilina. Foi confirmada a presença do gene mecA em apenas um isolado oxacilina resistente. No teste de disco difusão, 58,3% das enterobactérias foram resistentes à amoxicilina + ácido clavulânico e à cefotaxima, 50% à ceftazidima e ceftriaxona, e 33,3% à amoxicilina. Ainda entre os isolados Gram-negativos, não houve a expressão fenotípica de isolados ESBL. O presente trabalho expôs a presença de microrganismos resistentes a antibióticos em lhamas que não tiveram contato prévio com essas drogas, além da presença do gene mecA em um dos animais. O conhecimento da microbiota bacteriana de diferentes espécies animais tem se tornado cada vez mais importante. Tal relevância se deve à possibilidade de esses microrganismos serem compartilhados entre os animais, os humanos e até mesmo o meio ambiente.*

Palavras-chave: Lhamas, mecA gene, Saúde Única, Resistência

ACKNOWLEDGMENTS

We are thankful to UNIPAR and the Araucária Foundation (Institutional Program for Basic and Applied Research and Pro-Equipment Program) for funding this research, to CAPES for concession of the PROSUP school fee and CNPq (CP 09/2020) - Research Productivity Grants.

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