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Identification and Antimicrobial Resistance Profile in Bacteria Isolated from Brown Aracari (Pteroglossus castanotis australis) – Case Report

Identificação e Perfil de Resistência Antimicrobiana em Bactérias Isoladas de Araçari-Castanho (Pteroglossus castanotis australis) – Relato de Caso

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ABSTRACT

Studies involving Brown Aracari (Pteroglossus castanotis australis) are scarce due to the difficulty in studying free-living species without human interference, the works available in the literature are generally on animals in captivity. Free-living wild birds can be considered reservoirs of microorganisms carrying bacterial multi-resistance, which can become a serious problem in terms of unique health. The aim of this study was to identify the microorganisms and evaluate the profile of resistance to conventional antimicrobials in a free-living Brown Aracari (P. c. australis) in a border region in the state of Paraná, Brazil.

Keywords: Wild animal; Antimicrobial; Wild life; Bacteria.

RESUMO

Estudos envolvendo o Araçari-Castanho (*Pteroglossus castanotis australis*) são escassos devido à dificuldade em estudar espécies de vida livre sem interferência humana, os trabalhos disponíveis na literatura são geralmente sobre animais em cativeiro. Aves silvestres de vida livre podem ser consideradas reservatórios de microrganismos portadores de multirresistência bacteriana, o que pode se tornar um sério problema em termos de saúde única. O objetivo deste estudo foi identificar os microrganismos e avaliar o perfil de resistência aos antimicrobianos convencionais em um Araçari-Castanho (*P. c. australis*) de vida livre em uma região de fronteira no estado do Paraná, Brasil.

Palavras-chave: Animal selvagem; Antimicrobiano; Bactérias; Vida livre.

INTRODUÇÃO

Araçaris are birds of the Ramphastidae family, members of the Piciformes order, located in a neotropical region, distributed from southern Mexico to northern Argentina, present in all Brazilian biomes with greater diversity in the Amazon region. The bird is characterized by its elongated beak in relation to the body, vibrant colors of its feathers, with reddish, yellow, brown, black and green tones (Oliveira et al., 2019). The Brown Aracari (Pteroglossus castanotis australis) has ecological importance due to its seed dispersal potential, playing a key role in reforestation in different regions (Oliveira et al., 2019). The populations of Brown Aracaris are under anthropogenic pressure, due to changes caused by human beings in the environment, construction of highways, urban expansion and illegal trade. These birds have low reproductive success in captivity, thus, individuals on display or in possession in Brazil come from free life (Junior, 2012). They are birds of diurnal habits, nest in holes or crevices in trees, as well as in ravines and termite mounds. four eggs, with an incubation period of about 18 days (Helmut, 1997; Jennings, 2001).

The aim of this study was to identify isolated bacteria and analyze the antimicrobial resistance profile of a free-living Araçari (P. c. australis) and the concern with the transmission of resistant strains with zoonotic potential to humans, the environment and others animal species, contributing to the scientific literature and unique health.

CASE REPORT

The free-living Brown Aracari (*Pteroglossus castanotis australis*) was sent to the Veterinary Hospital of the Universidade Paranaense (UNIPAR), after a collision with an unidentified vehicle on the central avenue of the municipality of Umuarama (Parana, Brazil), in an attempt to resuscitate and stabilization, which did not resist due to the severity of the trauma. The geographic coordinates where the animal was located are: 23°45'23.5"S, 53°17'23.3"W. The animal was sent to the Department of Veterinary Pathology of the Department of Veterinary Medicine at Universidade Paranaense (UNIPAR) for identification of the species, collection of material and later sent to the Laboratory of Preventive Medicine and Public Health in the Postgraduate Program in Animal Science with Emphasis on Bioactive Products at Universidade Paranaense (UNIPAR) for specific analyses.

The Brown Aracari was a male weighing 258.20 grams, measuring 34.5 cm in length from the skull to the tip of the tail, with 4.7 cm in length from the skull from the base of the nasal bone to the occipital crest, 9.7 cm in length. beak length and 3 cm width, wings span of 47 cm, chest circumference of 5.2 cm and pelvic circumference 4.1 cm, lower limbs 7.2 cm in length from the femur to the distal phalanx. Age from 8 months to 1 year was determined through

comparative sexual dimorphism, body measurements, physiological characteristics of the species and based on Brazilian and foreign Ornithological literature (Helmut, 1997). The color of the feathers was greenish-black on the dorsum of the chest and tail, with reddish tones in the pelvic region, the ventral region of the chest in yellow with a red band dividing the thoracoabdominal portion, the cranial feathers of brown color around the eyes and nape, being black on top of the nasal bone forming a collar in the neck, the characteristics of the beak were black in the lower maxilla and yellow in the nasal region and upper maxilla (Figure 1).

Figure 1. Brown Aracari (Pteroglossus castanotis australis). Veterinary Pathology Sector from the Department of Veterinary Medicine at the Universidade Paranaense (UNIPAR).

Source: Elaboration of the authors, 2023.

Biological samples were collected from the oral cavity, ocular, cloaca and ventral and dorsal skin with a sterile plastic swab in a stuart transport medium (Absorve®, Brazil) in a rotating movement at the corresponding location and sent under refrigeration to the Laboratory of Preventive Medicine and Health Public (UNIPAR).

In the laboratory, the swabs containing the samples were inserted into tubes containing 3 mL of Brain Heart Infusion (BHI) medium and incubated at 37°C for 24 hours. After this period, the cultures obtained were streaked on plates containing Blood Agar and incubated at 37°C for 24

hours to isolate Gram-positive and Gram-negative aerobic bacteria. The isolated colonies on each plate were subcultured in BHI medium, incubated at 37°C for 24 hours and stored in BHI with 10% glycerol at a temperature of -20°C for conservation (Quinn et al., 2005). Each isolate was submitted to analysis of macroscopic characteristics, Gram stain and specific biochemical tests, in order to define the bacterial genus (Quinn et al., 2005). Staphylococcus spp. were submitted to the analysis of macroscopic characteristics, Gram stain and specific biochemical tests, in order to define the bacterial genus (Quinn et al., 2005). The identification of bacteria belonging to the Enterobacteriaceae family was performed through a set of biochemical tests included in the "Kit for Enterobacteriaceae" (NewProv®, Brazil), according to the manufacturer's recommendations (Quinn et al., 2005).

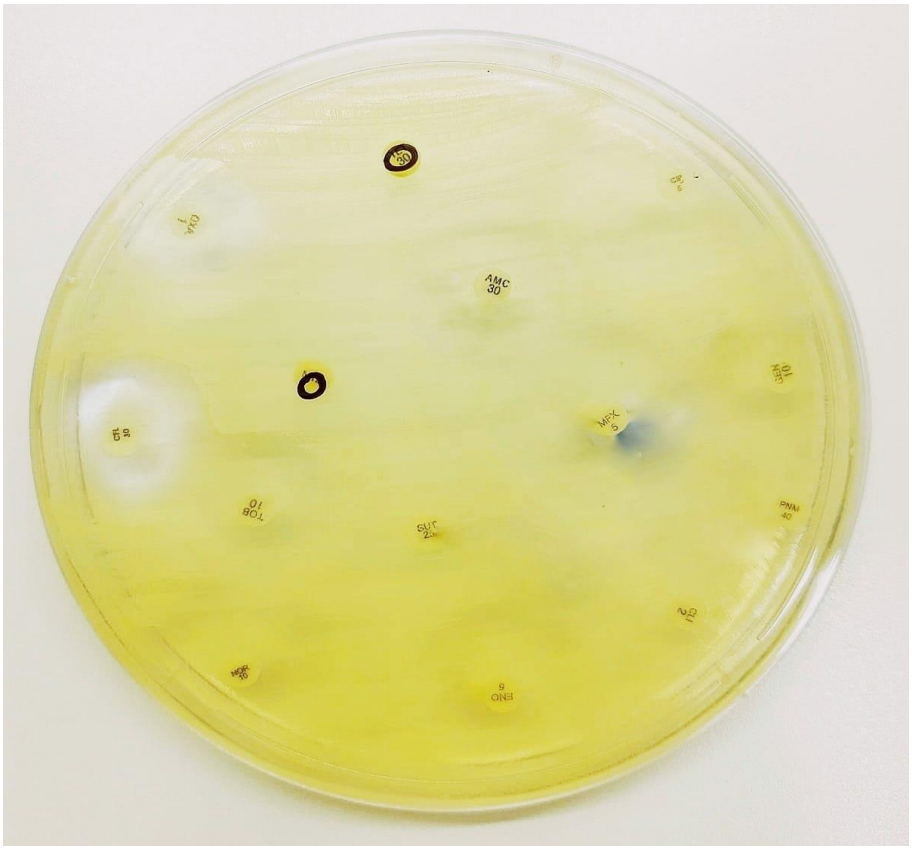
The antimicrobial susceptibility profile was evaluated by the agar diffusion disk method, in accordance with the recommendations of the Brazilian Committee on Antimicrobial Susceptibility Testing (BrCast, 2021). Discs tested for Gram-positive bacteria were: Ampicillin (10 µg), amikacin (30 µg), cephalotin (30 µg), cefotaxime (30 µg), ciprofloxacin (5 µg), clindamycin (2 µg), enrofloxacin (5 µg), gentamicin (10 µg), norfloxacin (10 µg), oxacillin (1 µg), penicillin (10 IU), sulfamethoxazole + trimethoprim (25 µg), tetracycline (30 µg). For Gram-negative bacteria, the discs tested were: Amoxicillin + clavulanic acid (30 µg), nalidixic acid (30 µg), amoxicillin (10 µg), amikacin (30 µg), ampicillin (10 µg), cephalothin (30 µg) , ceftiofur (30 µg), cefotaxime (30 µg), ceftriaxone (30 µg), cefepime (30 µg), cefoxitin (30 µg), ceftazidime (30 µg), ciprofloxacin (5 µg), chloramphenicol (30 µg), enrofloxacin (5 µg), ertapenem (10 µg), gentamicin (10 µg), imipenem (10 µg), sulfamethoxazole + trimethoprim (25 µg), tetracycline (30 µg), tobramycin (10 µg), meropenem (10 µg), moxifloxacin (5 µg) and norfloxacin (10 µg). A double-disk synergy test was performed in which disks containing cefotaxime (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg) and aztreonam (30 µg) were placed 20 mm from a disk containing amoxicillin + clavulanate (20/10 µg). Any increase or distortion of the inhibition zone of one of the antimicrobials towards the amoxicillin + clavulanic acid disk was considered suggestive for the production of extended-spectrum beta-lactamase (ESBL) (Brun-Buisson et al., 1987).

Bacterial growth was observed in all samples collected and through microbiological analysis Staphylococcus spp. negative coagulase in oral and skin samples. The enterobacteria Serratia marcescens and Serratia liquefaciens were observed in ocular, oral, cloaca and skin samples.

Antimicrobial susceptibility tests revealed multi-resistance in all microorganisms (Figure 2). For Staphylococcus spp. negative coagulase resistance was observed in all antimicrobials tested, except for cephalotin and oxacillin, enterobacteria showed high levels of resistance, namely: nalidixic acid, ampicillin, amikacin, amoxicillin + clavulanic acid, amoxicillin, cefoxitin,

gentamicin, tobramycin and moxifloxacin. The phenotypic test for detection of ESBL-producing strains was negative.

Figure 2. Antimicrobial multiresistance in brown Aracari (*Pteroglossus castanotis australis*).



Source: Elaboration of the authors, 2023.

DISCUSSION

Identification of *Staphylococcus* spp. occurred in all collected samples. These results corroborate with studies carried out in Minas Gerais, Brazil, where the main etiologies diagnosed in Piciformes birds are of the *Staphylococcus* spp. or of traumatic origin due to collisions (Dobbin *et al*., 2005**)**. Studies of the bacterial microbiota of clinically healthy free-living birds are essential for understanding the epidemiology of diseases that can affect their populations, similar species and humans, the occurrence and identification of these bacteria are of importance for public health (Dobbin *et al*., 2005**)**. Bacteria of the *Staphylococcus* spp. have already been isolated in cloacal samples of wild birds, being commonly isolated in this body region, the identification of this etiological agent is relevant due to the potential to cause joint infections in birds (Koneman, 2001). The multidrug resistance observed in *Staphylococcus* spp. isolates is also reported by authors in

several species of free-living birds, being the genus with the highest percentage of resistance to conventional antimicrobials (Livermore *et al*., 2001; Sayah *et al*., 2005).

In Brazil, high rates of antimicrobial resistance have been identified in microbiological samples of wild birds from the Atlantic Forest, which have possibly never received antimicrobial treatment (Nascimento *et al*., 2003**)**. Studies in order to understand the way in which these animals have a multiresistant microbiota would provide fundamental information for the development of alternative mechanisms in an attempt to control and/or treat these animals.

The enterobacteria *Serratia marcescens* and *Serratia liquefaciens* were isolated from ocular, oral, cloacal and skin samples. These microorganisms are reported to be part of the intestinal tract of free-range birds and can become opportunistic agents for infections when found elsewhere (Livermore *et al*., 2001). The isolates of *Serratia marcescens* and *Serratia liquefaciens* showed multi-resistance to different classes of antimicrobials tested, such as aminoglycosides, cephalosporin, penicillins and fluoroquinolone, except for ciprofloxacin. Studies have already observed that bacteria from the microbiota of wild birds have a lower rate of resistance to ciprofloxacin (Livermore *et al*., 2001). Collaborating with the information from this scientific study. In this scientific article, the antimicrobials tested for Gram-negative microorganisms, imipenem, meropenem and ertapenem, were found to reach the threshold of sensitivity according to the Brazilian Committee on Antimicrobial Susceptibility Testing (BrCast, 2021). This information is compatible with the findings of a study carried out with free-living bird cloacal swabs, where the enterobacteria were susceptible to imipenem (Santos *et al*., 2010). Demonstrating the importance and need for studies involving free-living animals, to understand and attempt to elucidate the occurrence of these multi-resistance in the exclusive antimicrobial classes in hospitals.

Studying populations of wild free-living birds, which are not normally exposed to antimicrobials, can be a way to understand antimicrobial resistance (Kümmerer, 2004). The collection of environmental material, monitoring of animal species and groups can favor research on resistance in free-living species. Theories about the development of resistance in free-living birds are discussed by science and may be through contact with faeces from domestic animals during periods of migration, direct contact with humans due to the advance of urbanization in natural areas of these animals and feeding human food waste (Santos *et al*., 2010).

It should be emphasized that antimicrobials used in medicine and veterinary medicine are not fully absorbed by patients, most of these drugs are excreted, contaminating the soil and water. It is suggested that part of the resistance found in free-living animals, probably never medicated, is a consequence of residual occurrence in the environment, together with human contact and between free-living animals (Kümmerer, 2004).

The results of this work demonstrated the presence of *Staphylococcus* spp. negative coagulase and multiresistant enterobacteria in ocular, oral, cloacal and skin samples of a free-

living Brown Aracari (*Pteroglossus castanotis australis*). These results indicate that free-living wild birds can be considered reservoirs of microorganisms carrying bacterial multi-resistance, which can become a serious problem in terms of unique health, due to the close interaction with other species of wild animals, direct contact with water and soil, enabling the transfer of these strains with humans.

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