

Cloacal enterobacteria isolated from captive roadside hawks (*Rupornis magnirostris*, GMELIN, 1788) and their antimicrobial susceptibility profile

Enterobactérias isoladas da cloaca de Gaviões-carijós (Rupornis magnirostris, GMELIN, 1788) cativos e seu perfil de susceptibilidade a antimicrobianos

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Abstract

Knowledge of the enterobacteria present in the roadside hawk can bring about an understanding of infectious diseases that can affect this bird, as well as other animals and/or humans, while also adding information of great ecological importance. Thus, the aim of the study was to determine the enterobacteria present in the cloaca of captive roadside hawks and antimicrobial susceptibility profile. Initially, cloacal samples from nine specimens were collected with the aid of swabs. Samples were placed in petri dishes with MacConkey agar, Hektoen agar, EMB agar and *Salmonella-Shigella* (SS) agar and incubated for 24 h at 35°C. After incubation, the microorganisms were submitted to biochemical testing to confirm the presence of enterobacteria. Thereafter, the susceptibility profile of bacteria to antimicrobial agents was evaluated by a disk diffusion test according to the *Clinical and Laboratory Standards Institute* (CLSI). *Escherichia coli* (77.8%), *Klebsiella oxytoca* (11.1%), *Klebsiella pneumoniae* (11.1%) and *Salmonella* spp. (55.6%) were isolated from the collected samples. Among the isolates, some bacteria showed resistance to up to three antimicrobial agents. This study has brought greater insight about the enterobacteria present in the roadside hawk (*Rupornis magnirostris*), exhibiting a significant percentage of enterobacteria important to public health; also, it showed the occurrence of strains with resistance profile to antimicrobial agents.

Keywords: Roadside hawk. Microbiology. Enterobacteria. Birds.

Resumo

O conhecimento das enterobactérias presentes em Gavião-carijó pode trazer uma compreensão sobre as doenças infecciosas que podem acometer essa ave, como também outros animais e/ou humanos, além de trazer mais informações sobre essa espécie de grande importância ecológica. Desta forma, o objetivo do estudo foi determinar as enterobactérias presentes na cloaca de Gaviões-carijós cativos e seu perfil de susceptibilidade a antimicrobianos. Inicialmente, foram coletadas amostras cloacais de nove espécimes com o auxílio de swabs. As amostras foram plaqueadas em ágar MacConkey, ágar Hektoen, ágar EMB e ágar SS e incubadas por 24 h a 35°C. Após incubação, as colônias foram submetidas às provas bioquímicas para confirmação da presença de enterobactérias. Posteriormente, o perfil de susceptibilidade das bactérias frente a agentes antimicrobianos foi avaliado através do teste da difusão em disco de acordo com o *Clinical and Laboratory Standards Institute* (CLSI). *Escherichia coli* (77,8%), *Klebsiella oxytoca* (11,1%), *Klebsiella pneumoniae* (11,1%) e *Salmonella* spp. (55,6%) foram isoladas das amostras coletadas. Dentre as cepas isoladas, algumas apresentaram resistência a até três antimicrobianos. O presente estudo trouxe um maior conhecimento sobre as enterobactérias presentes no Gavião-carijó (*Rupornis magnirostris*), mostrando um percentual significativo de enterobactérias de importância na saúde pública, evidenciando também a ocorrência de cepas com perfil de resistência a agentes antimicrobianos.

Palavras-chave: Gavião-carijó. Microbiologia. Enterobactéria. Patologia aviária.

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Introduction

The roadside hawk (*Rupornis magnirostris*) is a wild bird of prey belonging to the Accipitriformes order. This animal is found in a wide variety of environments (TORTATO, 2009) and has a diversified diet, typical of an opportunistic hunter (PANASCI; WHITACRE, 2000). It has an important ecological role as a top predator; it is responsible for maintaining and operating in communities to which they belong (RICKLEFS; MILLER, 2000). It is believed that wild animals in captivity are the source of 70% of emerging diseases, including increased resistance to antimicrobials (AHMED et al., 2007). According to Curcino et al. (2009), the roadside hawk is well adapted to urban centers and their population tends to progressively increase (IUCN, 2013). This opportunity for contact with humans and other animals can bring risks to public health through contamination by pathogens (CUBAS et al., 2006).

The enterobacteria of wild birds are still not much studied. The intestinal tract of predators has a high density of bacteria, including strict anaerobic and facultative anaerobes (SORUM; SUNDE, 2001). The proliferation of undesirable microorganisms can cause diarrhea, infection, and weight loss of the animal which may lead to death (ISOLAURI et al., 2004). In addition, the inappropriate use of antimicrobial agents exercised a great selective pressure, favoring the emergence of bacterial strains with resistance to antimicrobial agents, aggravating the problem of public health and making studies related to antimicrobial susceptibility relevant (GIBBS et al., 2007).

Thus, this study aimed to identify enterobacteria isolated from the cloaca of captive roadside hawks (*Rupornis magnirostris*) and analyze the susceptibility profile of these bacteria to antimicrobial agents.

Nine roadside hawks (five males and four females) were obtained from the Pernambuco Wild Animal Screening Center (CETAS) - IBAMA (Brazilian Environment and National Resources Institute) of Pernambuco. Permission from the Chico Mendes Institute for the Conservation of Biodiversity (ICMBio) was presented to acquire the specimens and was granted by the Ethics Committee on Animal Use (CEUA) and the Federal University of Pernambuco (UFPE) Center for Biological Sciences (CCB), under authorization process number 23076.018906 / 2013-25.

The collection of cloacal samples was carried out using sterile swabs which were kept in a brain-heart infusion broth (BHI) and tetrathionate broth for 24 h at 35°C. Afterward, samples were placed in petri dishes with MacConkey agar, Hektoen enteric agar, Salmonella-Shigella (SS) agar and eosin methylene blue (EMB) agar and reincubated for over 24 h at 35°C. After the last incubation period, the samples that showed microbial growth compatible with enterobacteria were submitted to biochemical tests, such as lactose fermentation, cytochrome oxidase, triple sugar iron (TSI), lysine and ornithine decarboxylase, hydrogen sulfide (H₂S) and carbon dioxide (CO₂) production on TSI agar, indole production, citrate, urease and motility to confirm the presence of these enterobacteria.

All of the bacteria were submitted to a disk diffusion test to evaluate the susceptibility profile to antimicrobial agents: ceftriaxone (30 µg), tobramycin (10mg), penicillin G (10U), imipenem (10 mg), amikacin (30 µg), ampicillin (10 µg), gentamicin (10mg), cephalothin (30 µg) and ciprofloxacin (0.5 mg) according to the *Clinical and Laboratory Standards Institute* (CLSI). The resistance or sensitivity profiles were expressed following the CLSI (2014) cutoff points.

After analysis of the samples from the nine animals, four enterobacteria were isolated with 14 strains identified (Table 1). Seven animals exhibited *Escherichia coli* (77.8%), one animal had *Klebsiella oxytoca* (11.1%), another, *Klebsiella pneumoniae* (11.1%) and five animals exhibited *Salmonella* spp. (55.6%). As expected, 77.8% of the animals exhibited *Escherichia coli*. This bacterium is frequently isolated

from the intestinal tract of wild birds and may, under certain conditions, cause infectious processes (ALMEIDA et al., 2005). In studies conducted by Santos et al. (2010), cracid cloacal samples exhibited a frequency of 70.5%, as well as with Steele et al. (2005) with fecal samples from gulls and seabirds showing a frequency of 71% and 90%, respectively.

Table 1 – Cloacal enterobacteria isolated from swabs samples of the roadside hawks (*Rupornis magnirostris*) – CETAS-IBAMA-PE – 2015

Roadside rawk	<i>Escherichia coli</i>	<i>Klebsiella oxytoca</i>	<i>Klebsiella pneumoniae</i>	<i>Salmonella</i> spp.
Animal 1	+	+	-	+
Animal 2	-	-	+	+
Animal 3	+	-	-	-
Animal 4	-	-	-	+
Animal 5	+	-	-	-
Animal 6	+	-	-	+
Animal 7	+	-	-	+
Animal 8	+	-	-	-
Animal 9	+	-	-	-
Total (%)	77.8	11.1	11.1	55.6

+: Presence; -: Absence.

According to the results obtained in this study, the genus *Salmonella* was the second most isolated probably because these animals lived in captivity. This genus is the most frequently isolated in outbreaks in several countries and is considered a major health care system problem because of the high incidence, high morbidity and, above all, the difficulty in controlling it (CHOMEL et al., 2007).

Beside the bacteria previously mentioned, other species were identified, such as *Klebsiella oxytoca* and *Klebsiella pneumoniae*. Among the species of this genus, these are the most commonly involved in disease processes in birds (CUBAS et al., 2006). These enterobacteria, along with others, are identified in studies as causing enteritis in birds, as well as ostriches (*Struthio camelus*) (HUCHZERMEYER, 2000).

It is important to mention that even some of enterobacteria isolated in our study may be part of the group of normal microorganisms in this anatomical

site of these animals. In some situations, they can become opportunistic pathogens (GIBBS et al., 2007); in particular, *Salmonella* spp., which has been described as a frequent cause of food poisoning outbreaks (CHOMEL et al., 2007).

The results of antimicrobial susceptibility testing are described in Table 2. All isolates were sensitive to gentamicin, tobramycin and amikacin (aminoglycoside). *K. oxytoca* showed sensitivity to all antibiotics. *E. coli* and the genus *Salmonella* were sensitive to ceftriaxone and imipenem, while *K. pneumoniae* exhibited a resistant profile. In addition, the lowest percentages of resistance against ampicillin, cephalothin and ciprofloxacin were observed.

Ahmed et al. (2007), in studies carried out with captive animals, found 21% of *E. coli* strains resistant to one or more antibiotics, especially ampicillin and the group of cephalosporins. These results were also reported by Vargas et al. (2010) with Barranquilla zoo

birds which were different from our work. Resistance to ampicillin and cephalothin (cephalosporin) in 14.3% of *E. coli* strains was found.

It was also noted that *K. pneumoniae* strains in studies performed by Ahmed et al. (2007) and Vargas et al. (2010) had 100% ampicillin resistance and this result was also found in the present study, because this bacterium is intrinsically resistant to ampicillin (PFALLER; SEGRETI, 2006).

Santos et al. (2010) also showed a low level of resistance to ciprofloxacin in cracids emphasizing that bacteria of fecal microbiota of wild birds had a lower rate of resistance to this antimicrobial agent.

Regarding resistance profile to more than one antibiotic, one *E. coli* strain exhibited resistance to ampicillin, cephalothin and ciprofloxacin, one *K. pneumoniae* strain was resistant to ciprofloxacin, ceftriaxone, and imipenem and one *Salmonella* spp. strain presented simultaneous resistance to ampicillin and cephalothin. Other authors already isolated

enterobacteria strains from captive birds with resistance to more than one antimicrobial agent (MARIETTO-GONÇALVES et al., 2010; SANTOS et al., 2010). This resistance can be characterized by the fact that roadside hawks lived in captivity and were in contact with humans (GIBBS et al., 2007).

Thus, this study has brought about greater insight into the enterobacteria of the roadside hawk (*Rupornis magnirostris*), a widely distributed bird, but not much investigated. The roadside hawk had a high percentage of enterobacteria important for public health, such as *Salmonella* spp. In addition, some of the bacteria isolated in this study exhibited a multidrug resistance profile to antimicrobial agents and this result supports further host/parasite studies.

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Table 2 – Antimicrobial susceptibility profile of cloacal enterobacteria of the roadside hawk (*Rupornis magnirostris*) – CETAS-IBAMA-PE – 2015

Antimicrobial agents	<i>Escherichia coli</i>				<i>Klebsiella oxytoca</i>				<i>Klebsiella pneumoniae</i>				<i>Salmonella spp.</i>			
	Resistant		Sensitive		Resistant		Sensitive		Resistant		Sensitive		Resistant		Sensitive	
	n	%	n	%	n	%	N	%	N	%	n	%	n	%	n	%
Penicillins																
-Penicillin	4	57.1	3	42.9	0	0	1	100	1	100	0	0	2	40	3	60
-Ampicillin	1	14.3	6	85.7	0	0	1	100	1	100	0	0	1	20	4	80
1st Generation Cephalosporin																
-Cephalothin	1	14.3	6	85.7	0	0	1	100	1	100	0	0	1	20	4	80
3rd Generation Cephalosporin																
-Ceftriaxone	0	0	7	100	0	0	1	100	1	100	0	0	0	0	5	100
Carbapenems																
-Imipenem	0	0	7	100	0	0	1	100	1	100	0	0	0	0	5	100
Aminoglycoside																
-Gentamicin	0	0	7	100	0	0	1	100	0	0	1	100	0	0	5	100
-Tobramycin	0	0	7	100	0	0	1	100	0	0	1	100	0	0	5	100
-Amikacin	0	0	7	100	0	0	1	100	0	0	1	100	0	0	5	100
Fluoroquinolone																
-Ciprofloxacin	1	10	7	90	0	0	1	100	0	0	1	100	1	20	4	80

n: number of samples.

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