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Antimicrobial susceptibility profile of enterobacteria isolated from wild grey-breasted parakeets (*Pyrrhura griseipectus*)[1]

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ABSTRACT.- Beleza A.J.F., Cardoso Maciel W., Carreira A.S., Marques A.R., Nunes F.P., Raso T.F., Horn Vasconcelos R., Teixeira R.S.C. 2021. **Antimicrobial susceptibility profile of enterobacteria isolated from wild grey-breasted parakeets (*Pyrrhura griseipectus*).** *Pesquisa Veterinária Brasileira 41:e06696, 2021.* Setor de Estudos Ornitológicos, Faculdade de Veterinária, Universidade Estadual do Ceará, Av. Paranjana 1700, Fortaleza, CE 60740-903, Brazil. E-mail: jacksonfortemv@gmail.com

The grey-breasted parakeet (*Pyrrhura griseipectus*) is an endangered psittacine species that have been affected by illegal trade and deforestation. Currently, this endemic species is only found in three areas in Ceará state, in Brazil. This study aimed to investigate the frequency and diversity of Enterobacteriaceae in wild adult grey-breasted parakeets and determine their susceptibility to antimicrobial agents. Cloacal swab samples were collected from 27 individuals and environmental swabs (drag swabs) from five nests used by these birds. Twenty-seven strains from nine species of Enterobacteriaceae were recovered from cloacal swabs, and the most prevalent bacteria strains were *Hafnia alvei* (22%) and *Pantoea agglomerans* (22%). From environmental nest samples, seven strains from three bacterial species were isolated, being the *P. agglomerans* the most frequent species (100%). Twenty-two of the 27 isolates (81.4%) exhibited antibiotic resistance, varying from one to eight of the 12 antimicrobials commonly used. Resistance to amoxicillin was the most prevalent (70.4%), followed by azithromycin (22.2%) and ceftriaxone (18.5%). None of the strains were resistant to gentamicin, tobramycin, ciprofloxacin or tetracycline. The *H. alvei* was the main species presenting multidrug resistance, including resistance against meropenem, which is an important finding. These results could provide interesting information on the health of these endangered wild grey-breasted parakeets. They could also indicate that the obtained isolates are part of a group of bacteria that are typical components of the enteric microbiota of birds, which present elevated rates of resistance to amoxicillin.

INDEX TERMS: Enterobacteria, wild grey-breasted parakeets, *Pyrrhura griseipectus*, antimicrobial resistance, conservation, psittacine birds, threatened species, wildlife animals.

RESUMO.- [**Perfil de susceptibilidade antimicrobiana de enterobactérias isoladas de periquitos-de-cara-suja (*Pyrrhura griseipectus*).**] O periquito-de-cara-suja (*Pyrrhura griseipectus*) é uma espécie de psitacídeo considerado pela IUCN como ameaçado de extinção, resultado do comércio ilegal e do desmatamento. Atualmente, essa espécie endêmica é encontrada apenas em três áreas no estado do Ceará, Brasil. O objetivo deste estudo foi investigar a frequência e a diversidade de Enterobacteriaceae em periquitos de peito cinza adultos selvagens e determinar sua suscetibilidade a agentes antimicrobianos. Amostras de suabes cloacais foram coletadas de 27 indivíduos e de suabes ambientais (suabes de arrasto) de cinco ninhos utilizados por essas aves. Vinte

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e sete cepas de nove espécies de Enterobacteriaceae foram isoladas a partir de suabes cloacais, sendo as cepas bacterianas mais prevalentes *Hafnia alvei* (22%) e *Pantoea agglomerans* (22%). Das amostras ambientais de ninhos foram isoladas sete linhagens de três espécies bacterianas, sendo *P. agglomerans* a espécie mais frequente (100%). Vinte e dois dos 27 isolados (81,4%) exibiram resistência a antibióticos, variando de um a oito dos 12 antimicrobianos comumente usados. A resistência a amoxicilina foi a mais prevalente (70,4%), seguida por azitromicina (22,2%) e ceftriaxona (18,5%). Nenhuma das cepas era resistente à gentamicina, tobramicina, ciprofloxacina ou tetraciclina. *H. alvei* foi a principal espécie que apresentou resistência a múltiplas drogas e que também esteve associada a um outro achado relevante desta pesquisa, que foi a detecção de um caso de resistência ao meropenem. Esses dados fornecem informações relevantes sobre a saúde desses periquitos selvagens ameaçados e permite concluir que os isolados obtidos fazem parte de um grupo de bactérias que normalmente compõe a microbiota entérica das aves, sendo a amoxicilina envolvida em elevadas taxas de resistência.

TERMOS DE INDEXAÇÃO: Enterobactérias, periquitos-de-cara-suja, *Pyrrhura griseipectus*, resistência antimicrobiana, conservação, aves psitacídeos, espécies ameaçadas, animais selvagens.

INTRODUCTION

Pyrrhura griseipectus (Salvadori, 1900), commonly known as the grey-breasted parakeet, is a member of the order Psittaciformes, family Psittacidae, represented by parrots, cockatoos, macaws and parakeets. This species is endemic to northeastern Brazil and can be found in four regions of the state of Ceará as following: Maciço de Baturité; Quixadá; Ibaretama; and Canindé (BirdLife International 2018, Nunes 2018). More recently, a free-living group was discovered in the municipality of Conde, in the state of Bahia (Brasileiro 2019). This parakeet is usually found in humid forests in regions with an altitude 500m above sea level (Girão et al. 2008).

Recent data on a population survey of *P. griseipectus* have not yet been published. What is known, according to the Red List of the International Union for the Conservation of Nature (IUCN), the grey-breasted parakeet is still considered an endangered species, with a population that can range from 250 to 999 sexually mature individuals in the wild. Anthropogenic pressure on the Maciço de Baturité, especially at higher altitudes, has increased considerably in recent decades. The largest population of grey-breasted parakeets is found on this mountain. These anthropic actions generally lack sustainable practices, leading several spatial sectors of the Maciço de Baturité to a problematic state of degradation (Da Silva et al. 2016). Therefore, deforestation, illegal trade in wild animals, and agriculture in forests containing this species are the main threats that affect conservation and preservation (BirdLife International 2018).

The destruction of vegetation cover in the habitat of *P. griseipectus* has been verified over time. Consequently, there has been a decline in the population of these birds (Campos et al. 2014). This species breeds preferentially in forest edges (Licarião 2014), and some tree species are considered preferred choices for nesting, such as Albízia (*Albizia polycephala* (Benth.) Killip) and Ingá (*Inga* sp.) (Girão et al. 2008). With a predominance of young trees, regenerating environments tend to have few holes for birds to use as a breeding site (Gibbons & Lindenmayer 2002, Ranius et al. 2009, Manning et al. 2013). Due to the lack of natural holes in the Serra de Baturité, the "Associação de Pesquisa e Preservação de Ecossistemas Aquáticos" (AQUASIS) developed a program using an artificial environment, called nest-boxes caixas-ninho, as a tool that encourages the reproduction of these parrots. The installation of nests in an appropriate location, associated with the possibility of feeding in the surroundings, has boosted the reproductive success of these birds (Nunes 2017).

Research involving free-living birds has become important in recent years, especially when involving the management and conservation of threatened species (Foldenauer et al. 2007, Limiñana et al. 2009). Due to the growing evidence that certain diseases can cause adverse impacts on these populations, it has been increasingly important to carry out health monitoring of these species (Deem et al. 2005).

Studies on the microbiota of free-living parrots are scarce, and little is known about the subject. Some studies suggest that colonization by enterobacteria is related to captivity, as their free-living enteric microbiota is composed of Gram-positive cocci and bacilli (Xenoulis et al. 2010, Waite & Taylor 2014). There is a need for greater elucidation about Gram-negative bacteria composing the enteric microbiota of free-living parrots. Also, it is vital to know about the health status of the grey-breasted parakeet (*P. griseipectus*) conservation. The present study aimed to investigate enterobacteria in cloacal samples of birds and the nests they use and to determine the phenotypic profile of antimicrobial sensitivity of the isolated bacteria.

MATERIALS AND METHODS

Study area. Free-range adult grey-breasted parakeets (*Pyrrhura griseipectus*) were evaluated in areas located in two municipalities in the state of Ceará, in Brazil: Guaramiranga and Ibaretama. Guaramiranga is located in a region known as Maciço do Baturité, an Environmental Protection Area ("Área de Proteção Ambiental" - APA), whose highest peak reaches 1115m in altitude, consisting of humid forests in the middle of a dry and hot region. The location used to capture parakeets in Ibaretama was Serra Azul, a region delimited by a residual massif, whose highest peaks can exceed 700m in altitude. At the foothill, there are areas where agropastoral activities modified the original vegetation. At some lower points on the mountain slope, there are spaces modified by human action, interspersed with areas of dense shrubby Caatinga. In the higher areas, there is the existence of *prickly* deciduous forest or arboreal Caatinga.

This study was authorized by the "Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis" (IBAMA), under protocol SISBIO No. 31847-6, and approved by the Local Ethics Committee for the use of animals at the "Universidade Estadual do Ceará" (UECE) (Protocol 4832011/2014).

Samples. Twenty-seven adult parakeets without clinical signs of disease were captured in the wild, 25 in Baturité and two in Ibaretama. In the Baturité area, AQUASIS used artificial wooden nest-boxes to assist in the parakeet breeding and conservation program. These nests were placed on several private properties close to the species' natural habitat. Usually, adult parakeets sleep inside communal nests, so the biological samples were collected at night through a cloacal smear. In addition, samples of drag swabs were also collected from the interior surface of the nest boxes.

In the Ibaretama area, there were no artificial nests, and the natural nests of parakeets were not accessible, as they are located on top of a rocky wall. Therefore, to capture the birds, two mist nets (12m x 2.5m and 35mm mesh) were set up close to the natural nest and observed at intervals of 20 minutes to verify the presence of individuals in the nets.

The census carried out by AQUASIS in 2017 revealed that the estimated natural population of parakeets in the Baturité sampling region was 314 individuals. In Ibaretama, the estimated population was only eight free-living individuals. Thus, the samples represented 8.4% of the total monitored population.

Biological samples were obtained using sterile cloacal swabs, which were stored in Stuart Transport Medium at room temperature, transported and sent within 48 hours to the "Laboratório de Estudos Ornitológicos" (LABEO-UECE) for further microbiological processing. After sampling, individuals were promptly returned to their nests or released to the natural environment.

Microbiological procedure. In the laboratory, samples were taken from Stuart Transport Media, placed in 5mL of peptone water and subsequently subjected to incubation. The temperature and incubation time was standardized at 37°C/24h for this and subsequent steps of microbiological processing. The 0.5mL aliquots of the peptone water samples were transferred to Brain-Heart Infusion (BHI) and Selenite-Cystine (SC) enrichment broths, and, subsequently, a volume of 0.05mL was transferred to Rappaport-Vassiliadis broth (RP). After incubation, the three enrichment broths were seeded onto plates containing brilliant green agar (BG), *Salmonella Shigella* agar (SS) and MacConkey agar (MC) and incubated again. Subsequently, the plates were observed for the presence of colonies with distinct morphological characteristics and, if there was growth, one of each profile in each plate were collected and inoculated into tubes containing Triple Sugar Iron agar (TSI). After the incubation period, to confirm the enterobacteria, a biochemical battery consisting of the following media was used: Lysine Iron Agar (LIA), SIM medium, Simmons citrate (CIT), Methyl Red (MR), Voges-Proskauer (VP), ornithine decarboxylase (ODC), urea, malonate, lactose, sucrose, mannitol, arabinose, rhamnose, raffinose, dulcitol, adonitol, inositol, and sorbitol (Procop et al. 2018).

Antimicrobial susceptibility profile of isolates. The isolates were subjected to an antibiogram using the Kirby-Bauer diffusion disk technique. The inhibition halos were read according to the Clinical and Laboratory Standards Institute (CLSI 2019). On the surface of Muller-Hinton agar, after sowing the solution (sample), discs with antimicrobials were dispensed, and the measurement of inhibition zones was performed after incubation at 37°C/24h. Twelve antimicrobials from seven pharmacological classes were tested: quinolones (nalidixic acid, 30μg); fluoroquinolones (ciprofloxacin, 5μg); aminoglycosides (gentamicin, 10μg and tobramycin, 10μg); tetracyclines (tetracycline, 30μg); macrolides (azithromycin, 15μg); sulfonamides (sulfamethoxazole + trimethoprim, 25μg); and beta-lactam (penicillin: amoxicillin, 10μg and amoxicillin + clavulanic acid 10μg, cephalosporins: ceftriaxone, 30μg and carbapenems: meropenem 10μg). Bacteria were considered multiresistant when resistant to at least three classes of antibiotics (Magiorakos et al. 2012). The *Escherichia coli* ATCC 25922 strain was used as a control sample.

RESULTS

Isolated bacteria. A total of 27 isolates belonging to nine species of eight genera of enterobacteria were obtained from the cloacal swabs of adult grey-breasted parakeets. The most prevalent bacteria were *Hafnia alvei* and *Pantoea agglomerans*, both with rates of 22.2% (6/27). The *Escherichia coli* was the third most isolated bacterium, with a prevalence of 18.5% (5/27), followed by *Klebsiella pneumoniae*, with 11.1% (3/27).

Of the five samples of drag swabs from the nest environment, seven isolates were obtained, with three different bacterial species identified. *P. agglomerans* was the most isolated bacterium, with a frequency of 100% (5/5). *Cronobacter sakazakii* and *H. alvei* were identified only once (20.0%) in the five samples analyzed (Table 1).

Antimicrobial resistance. Regarding bacterial resistance, the highest rate of occurrence was observed with amoxicillin, with 70.4% (19/27). Even disregarding the cases of intrinsic resistance (*Citrobacter freundii*, *H. alvei* and *K. pneumoniae*), it was still the antibiotic with less effectiveness. In this case, 11 strains (73.3%) were still resistant, taking into account only a total of 15 investigated isolates.

Resistance to azithromycin and ceftriaxone was identified in 22.2% (6/27) and 18.5% (5/27) of the isolates, respectively (Table 2). Four antibiotics (gentamicin, tobramycin, ciprofloxacin and tetracycline) showed the best efficacy, as 100% of the investigated strains were susceptible. Nalidixic acid and meropenem also performed well (96.3% efficacy). One case of resistance to each of these antibiotics was observed in different strains of *H. alvei*. In the specific case of *E. coli*, amoxicillin was involved in the highest rates of resistance observed (60%), being detected in three of the five isolates.

Table 1. Prevalence of enterobacteria isolated from cloacal swabs and nests of grey-breasted parakeets (*Pyrrhura griseipectus*) in the state of Ceará, Brazil

Isolated bacteria	Prevalence in cloaca samples	Prevalence in nest-box isolate
*Citrobacter freundii**	7.4% (2/27)	0%
Cronobacter sakazakii	0%	20.0% (1/5)
*Enterobacter gergoviae**	3.7% (1/27)	0%
Escherichia coli	18.5% (5/27)	0%
Hafnia alvei	22.2% (6/27)	20.0% (1/5)
Klebsiella oxytoca	3.7% (1/27)	0%
Klebsiella pneumoniae	11.1% (3/27)	0%
Pantoea agglomerans	22.2% (6/27)	100.0% (5/5)
*Serratia rubidaea**	7.4% (2/27)	0%
Positive samples	81.5% (22/27)	100.0% (5/5)

* Isolated from the cloacal swabs of the two birds in the city of Ibaretama, in the state of Ceará. The other isolates were obtained from bird swabs and artificial nests where the species uses it for roosting in the city of Guaramiranga, Ceará.

Antimicrobial resistance related to azithromycin and ceftriaxone was also detected. Each case represented a rate of 20% of the analyzed *E. coli* strains. Regarding the bacteria isolated from the nest-box samples, only two strains were involved in cases of resistance, both linked to amoxicillin.

Only five of the 27 (18.5%) isolates were not resistant to the antibiotics tested. About 59.3% of the strains were susceptible to at least one class of antibiotics (Table 3). Multidrug resistance was observed in three strains (11.1%), all resistant to three classes of antibiotics (β-lactams + sulfonamides + macrolides). The same percentage (11.1%) also occurred for three other strains, which were resistant to two classes of antibiotics (β-lactams + macrolides). No *E. coli* isolate was resistant to multiple drugs, one strain was resistant to two classes of antibiotics, and another three to only one class.

DISCUSSION

In the present study, *Pantoea agglomerans* and *Hafnia alvei* were the most frequently isolated bacterial species in the cloacal swabs samples of adult grey-breasted parakeets. These two bacterial species can be found in soil and water. Indeed, *P. agglomerans* can be frequently isolated from plants, fruits and vegetables, presenting pathogenicity more frequently in plants (Scheutz et al. 2005, Segado-Arenas et al. 2012). In addition, it also occurs in vertebrates and invertebrates, but its clinical importance in animals is almost unknown (Dutkiewicz et al. 2016). Despite limited information about its occurrence in birds, *P. agglomerans* have been reported in healthy captive passerines, Psittaciformes, and other free-living bird orders (Lopes et al. 2015 Beleza et al. 2019, Carreira 2019). Gerlach et al. (1994) clarified that it is likely that the isolation of this microorganism in poultry feces can be related to the consumption of seeds containing this bacterium. Only when there is a high concentration of this agent in the food can toxicity problems occur. Some studies have reported *H. alvei* with domestic and wild birds (Hernandez et al. 2003, Giacopello et al. 2015, Beleza et al. 2019). However, reports on clinical conditions in birds with both bacteria are scarce in the literature. One of the rare cases occurred from the description of an outbreak in pullets of 18 weeks in Italy, whose clinical signs resembled those caused by *Salmonella* spp. (Proietti et al. 2004). In the present study, no bird showed clinical signs of the disease.

Table 2. Absolute and relative frequencies (%) of antimicrobial resistance of bacteria isolated from the cloaca of grey-breasted parakeets (*Pyrrhura griseipectus*) in the state of Ceará, Brazil

Bacteria (number of isolates)	Antibiotics										
	GEN	AZI	TOB	AMO	CEF	AMO + A. CLA	CIP	TET	SUL	A. NAL	MER
Citrobacter freudii (2)	0 (0.0%)	2 (100.0%)*	0 (0.0%)	2 (100.0%)*	0 (0.0%)	1 (50.0%)*	0 (0.0%)	0 (0.0%)	1 (50.0%)	0 (0.0%)	0 (0.0%)
Enterobacter gergoviae (1)	0 (0.0%)	0 (0.0%)*	0 (0.0%)	1 (100.0%)	0 (0.0%)	1 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Escherichia coli (5)	0 (0.0%)	1* (20.0%)	0 (0.0%)	3 (60.0%)	1 (20.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Hafnia alvei (7)	0 (0.0%)	2 (28.6%)*	0 (0.0%)	4 (57.1%)*	3 (42.9%)	1 (14.3%)*	0 (0.0%)	0 (0.0%)	1 (14.3%)	1 (14.3%)	1 (14.3%)
Klebsiella oxytoca (1)	0 (0.0%)	0 (0.0%)*	0 (0.0%)	1 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Klebsiella pneumoniae (3)	0 (0.0%)	0 (0.0%)*	0 (0.0%)	2 (66.6%)*	1 (33.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Pantoea agglomerans (6)	0 (0.0%)	0 (0.0%)*	0 (0.0%)	5 (83.3%)	0 (0.0%)	1 (16.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Serratia rubidaea (2)	0 (0.0%)	1 (50.0%)*	0 (0.0%)	1 (50.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (50.0%)	0 (0.0%)	0 (0.0%)
TOTAL (27)	0 (0.0%)	6 (22.2%)*	0 (0.0%)	19 (70.4%)	5 (18.5%)	4 (14.8%)	0 (0.0%)	0 (0.0%)	3 (11.1%)	1 (3.7%)	1 (3.7%)

GEN = gentamicin, AZI = azithromycin, TOB = tobramycin, AMO = amoxicillin, CEF = ceftriaxone, AMO+A.CLA = amoxicillin with clavulanic acid, CIP = ciprofloxacin, TET = tetracycline, SUL = sulfonamide, A. NAL = nalidixic acid, MER = meropenem; * Intrinsic resistance according to CLSI (2019).

Table 3. Resistance to multiple drugs of bacteria isolated from cloacal swabs from grey-breasted parakeets (*Pyrrhura griseipectus*) in the state of Ceará, Brazil

Antibiotic class numbers	Frequency of resistant isolates (%)	Identification of resistant bacterial strains (absolute frequency)	Antibiotic classes (absolute frequency of resistant strains)
0	18.5% (5/27)	EC(1), HA(1), KP(1), PA(1), SR(1)	-
1	59.3% (16/27)	EC(3), KP(2), PA(5), HA(4), KO (1),EG (1)	β-lactams (15) Quinolones (1)
2	11.1%(3/27)	CF(1), EC(1), HA(1)	β-lactams + macrolides (3)
3	11.1% (3/27)	CF(1), HA(1), SR (1)	β-lactams + sulfonamides + macrolides (3)

CF = *Citrobacter freundii,* EC = *Escherichia coli,* EG = *Enterobacter gergoviae,* HA = *Hafnia alvei,* KO = *Klebsiella oxytoca,* KP = *Klebsiella pneumoniae,* PA = *Pantoea agglomerans,* SR = *Serratia rubidaea.*

In fact, *Escherichia coli* was also among the most isolated microorganisms (18.5%), a lower percentage than Machado et al. (2018) found in their research on free-living *Pyrrhura griseipectus* juveniles. They obtained percentages of 65.8% of positive individuals. They also showed that this high rate could be explained by the accumulation of fecal material in the nest, which would increase the exposure of juveniles to fecal bacteria, unlike what occurs in adults and young individuals who live most of the time in less contaminated areas (Benskin et al. 2009). This microorganism and the other bacterial species isolated in this research are part of the normal parrot microbiota (Vaz et al. 2017). However, Xenoulis et al. (2010) could clarify that *E. coli* was more abundantly detected in captive-bred parrots than in wildlife, with a larger population of Gram-positives, such as *Staphylococcus saprophyticus*. This difference may result from factors such as the sampling location where the birds live and their diet (Waite & Taylor 2014). However, *E. coli*, despite its mutualism with the host, has strains with distinct virulence characteristics, classified into diarrheagenic and extraintestinal pathotypes (Croxen & Finlay 2010). Thus, pathogenic strains, when present in the birds' body, can, in certain circumstances, present themselves as harmful to health (Lopes et al. 2016).

Thus, *Klebsiella pneumoniae*, *Klebsiella oxytoca* and *Citrobacter freundii* are opportunistic and ubiquitous microorganisms (Bagley et al. 1978, Fernández et al. 2011, Davies et al. 2016). Despite having some clinical importance for birds, their isolation in feces does not necessarily harm the birds' health. Other research carried out with captive and free-living parrots reported *K. pneumoniae* and *C. freundii* isolated from the cloacal swabs of asymptomatic birds (Lopes et al. 2015, Machado et al. 2016, 2018). Compared to our results, Vaz et al. (2017) isolated a higher rate of *K. oxytoca* (22.7%) from cloacal swabs samples from red-tailed parrot (*Amazona brasiliensis*) chicks born in artificial wooden nests, also without signs of clinical abnormality. Although considered a likely member of the intestinal microbiota of animals, *C. freundii* can affect any avian species. It has been associated with septicemia and hepatitis, especially in young and immunosuppressed birds (Gerlach et al. 1994, Cubas & Godoy 2005). In addition, it has been identified as the principal agent of sudden death cases in two adult captive-bred parrots (Churria et al. 2014). Bacteria of the *Klebsiella* genus can also affect the health of free-range birds. It can cause infections restricted to the upper respiratory tract or systemic infections in adverse situations, especially in parrots (Gerlach et al. 1994).

The frequency of total enterobacteria isolated from cloacal swabs of adult parakeets was considered high (81.5%). Likewise, Machado et al. (2018), studying juvenile *P. griseipectus*, demonstrated that at least one strain of enterobacteria was isolated from each sample. Vaz et al. (2017) also detected similar bacterial species in apparently healthy free-living *A. brasiliensis* juveniles. These discoveries corroborate studies that show how these microorganisms are part of the intestinal microbiota of parrots and are not necessarily correlated with diseases. The parrot microbiota is composed of Gram-positive bacteria, although it is also normal to isolate Gram-negative bacteria, such as enterobacteria of the asymptomatic birds' cloaca. These enterobacteria can cause health problems in cases where the animals are subject to some condition of stress and subsequent immunosuppression (Gerlach et al. 1994).

The resistance rate to amoxicillin was 70.4% higher than other antibiotics, including azithromycin, which had the second-highest rate (22.2%). It is essential to consider that some of the isolated microorganisms have intrinsic resistance to amoxicillin; however, even disregarding these isolates, the total resistance rate for this antibiotic was still high (72.2%) - compared to the five *E. coli*, three (60.0%) showed resistance. Carreira (2019) evaluated the antimicrobial resistance of enterobacteria from cloacal swabs of several species of free-living birds captured in Fortaleza, in the state of Ceará, in Brazil. They found similar results regarding resistance to amoxicillin, which was the highest (73%), followed by azithromycin (54%). In addition, other studies have reported low susceptibility rates to azithromycin in strains isolated from parrot cloacal swabs in the state of Ceará. Lopes et al. (2015) evaluated parrots from wild bird traffic and found that the resistance rate was 91.3%. Likewise, a study with *P. griseipectus* from captivity in the same state of Ceará reported that none of the 14 isolates of enterobacteria showed susceptibility to this antibiotic (Machado et al. 2016). According to the Clinical and Laboratory Standards Institute (CLSI 2019), microorganisms of the Enterobacteriaceae family, except for *Salmonella* and *Shigella*, showed intrinsic *in vivo* resistance to azithromycin in humans, even if they were sensitive in the *in vitro* test. This fact may have a relationship with the resistance rate of the bacteria isolated from parakeets in this research. However, although macrolides have low activity on enterobacteria, compared to other drugs in this group, azithromycin has a higher basic character, which gives it a better membrane penetration (Gomes et al. 2017). It can be used to treat, for example, *E. coli* in case of diarrheal infections (Lübbert 2016).

In parrots, studies that evaluated the antimicrobial sensitivity of bacteria of the Enterobacteriaceae family do not show methodological uniformity concerning the bacterial and antimicrobial species evaluated. Furthermore, they are very scarce, and the few that exist are aimed at investigating *E. coli* (Lopes et al. 2015). Hidasi et al. (2013) evaluated *E. coli* from captive parrots from wild animals' illegal trade. They found high rates of resistance to amoxicillin (70.93%), tetracycline (69.19%) and ciprofloxacin (23.25%), which presented superior results to our study. A research carried out on birds from wild animal trafficking in the state of Ceará, Brazil, showed that *E. coli* obtained from the execution of cloacal smears on passerines and parrots had relevant rates of resistance to azithromycin, 40.8% and 91.3%, respectively (Lopes et al. 2015, Gaio et al. 2019). Machado et al. (2016) isolated *E. coli* in four of 27 cloacal swab samples of *P. griseipectus* in captivity, all of which were resistant to azithromycin. Similar to Machado et al. (2018), our study found low resistance rates to this antibiotic, which may be related to the lesser or absent contact with bacteria containing resistance genes or microbial agents than birds raised in captivity or coming from wild animal trafficking - since they are free-living birds. Despite the influence of intrinsic bacterial resistance, the most relevant mechanisms of resistance to azithromycin in Enterobacteriaceae are those encoded in mobile elements (Gomes et al. 2017).

The resistance detected in the *H. alvei* strain associated with meropenem was also one of the relevant data of this research. Scientific reports have shown that meropenem resistance in Enterobacteriaceae still behaves relatively low (Romero et al.

2015). However, Carbapenem-Resistant Enterobacteriaceae (CRE) have been increasingly reported worldwide (Magalhães & Soares 2018). Cases of resistance to carbapenems are more comprehensively reported in the scientific literature, usually associated with humans in a hospital environment (Lavagnoli et al. 2017, Mota et al. 2018, Palacios-Baena et al. 2020). It is occasionally possible to verify these reports in veterinary medicine, usually associated with domestic animals and, more rarely, wild animals (Chika et al. 2017, Sousa et al. 2019, Yu et al. 2020). In free-living animals, reports also exist (Guerra et al. 2014), but they are even more restricted.

Multidrug resistance was observed in three strains (11.2%); the relevance of this result is that these are free-living birds. In captive birds, it is easier to understand the occurrence of multidrug resistance, considering that they are generally related to the indiscriminate use of antibiotics or more significant contact with resistant strains from other birds (Matias et al. 2016, Siqueira et al. 2017). In parrots from trafficking, Hidasi et al. (2013) observed higher rates, as 23.5% of the samples were multiresistant to three antibiotic groups and 2.32% to four antibiotic groups. In captive cockatiels (an exotic species in Brazil), 30% of the isolates were resistant to seven or more antibiotics, and 59% of the strains were resistant to multiple drugs (Pontes et al. 2018). The results of resistance to one or more antibiotics in free-living *P. griseipectus* may suggest that at some point, these birds have had contact with resistant strains or antibiotic residues in the environment, considering that these birds are not subjected to antibiotic therapy. Although cases of multidrug resistance in free-living birds are less expected, Machado et al. (2018) identified cases of resistance to two, three, four and even seven classes of antimicrobials associated with *E. coli* strains isolated from free-living *P. griseipectus* juveniles in the Maciço de Baturité.

In free-living birds, there is the possibility of microorganisms acquiring resistance in contact with the material in the environment. Sándor et al. (2012) emphasized a possible long permanence of antibiotics in the environment, especially in lagoons, silt, marine sediments and aquaculture facilities. In addition to the permanence of antibiotics, it has also been observed that several antibiotics are excreted without modification (e.g., amoxicillin), making them very reactive, even after being eliminated from the body. It is interesting to consider that bacteria naturally resistant to antibiotics are widespread in the environment. This ancient and natural phenomenon is not related to the resistance arising from the modern selective pressure of clinical antibiotics (D'Costa 2011), which explains the resistance rates detected in free-living birds that have not had contact with antibiotics residues in the environment.

CONCLUSIONS

The profile of enterobacteria isolated from wild grey-breasted parakeets was composed mainly of *Hafnia alvei* and *Pantoea agglomerans*. The highest resistance rate was detected in amoxicillin.

Although only one occurrence of antimicrobial resistance to meropenem has been recorded, this is a relevant finding since such resistance is uncommon to be observed in isolates from free-living animals.

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