















# The occurrence of enterobacteria and the rate of resistance to antimicrobials in illegally trafficked *Sporophila passerines*

Ocorrência de enterobactérias e taxa de resistência a antimicrobianos em passeriformes do gênero *Sporophila* provenientes do tráfico de animais silvestres

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**Abstract:** We investigate the occurrence of enterobacteria and antimicrobial resistance in passerines of the genus *Sporophila* seized from illegal trafficking. A total of 35 birds, apparently healthy and from illegal breeding, were sent to the Laboratory of Ornithological Studies at the State University of Ceará (Fortaleza, Ceará, Brazil) by environmental agencies. Cloacal swabs were collected from each bird and subjected to traditional microbiological processing, using standard cultivation and bacterial-identification techniques. We conducted antimicrobial susceptibility testing using the disc-diffusion method. Twenty-three birds (65.7%) exhibited enterobacteria, with *Escherichia coli* (28.6%) and *Serratia liquefaciens* (25.7%) being the most common. Antimicrobial resistance in total isolates was observed most frequently in relation to ciprofloxacin (28.1%), followed by tetracycline (25.0%) and enrofloxacin (18.8%). The rate of resistance to *Serratia liquefaciens* was the highest (i.e., 66.6% of isolates). The birds' cloacal samples revealed several members of the Enterobacterales order, with *E. coli* and *S. liquefaciens* being the most prevalent. We also observed antimicrobial resistance to several drugs, as well as cases of multi-resistance. Antimicrobial resistance is clearly present in wild birds resulting from illegal keeping. These findings highlight the importance of considering antimicrobial-resistant bacteria in release programs to prevent these microorganisms from dispersing into the environment.

**Keywords:** *Escherichia coli*, *Serratia liquefaciens*, multi-drug resistance.

**Resumo:** O objetivo desta pesquisa foi investigar a ocorrência de enterobactérias em passeriformes do gênero *Sporophila*, provenientes do tráfico ilegal de animais, e analisar sua resistência antimicrobiana. Um total de 35 aves, aparentemente saudáveis e provenientes de criações ilegais, foram encaminhadas ao Laboratório de Estudos Ornitológicos da Universidade Estadual do Ceará (Fortaleza, Ceará, Brasil) por órgãos ambientais. Amostras obtidas a partir de suabes cloacais foram coletadas de cada ave e submetidas a processamento microbiológico tradicional, utilizando técnicas padrão

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de cultivo e identificação bacteriana. O teste de sensibilidade aos antimicrobianos foi avaliado pelo método de disco de difusão. Das amostras examinadas, 23 (65,7%) apresentaram a presença de enterobactérias, sendo *Escherichia coli* (28,6%) e *Serratia liquefaciens* (25,7%) as mais frequentes. A resistência antimicrobiana referente aos isolados totais foi observada com mais frequência em relação à ciprofloxacina (28,1%), seguida por tetraciclina (25,0%) e enrofloxacin (18,8%). Especificamente em relação à *Serratia liquefaciens*, a taxa de resistência foi a mais altas, atingindo 66,6% dos isolados. Com base nos resultados obtidos, podemos concluir que as amostras cloacais das aves apresentaram isolamento de diversos membros da ordem Enterobacterales, sendo *E. coli* e *S. liquefaciens* as mais frequentes. Também foi observada a ocorrência de resistência antimicrobiana a diversos fármacos utilizados, assim como casos de multiresistência, o que mostra que esse problema está presente em pássaros silvestres oriundo de manutenções ilegais. Isso ressalta a necessidade de considerar a presença de bactérias resistentes a antimicrobianos em programas de soltura, visando evitar a dispersão desses microrganismos no meio ambiente.

**Palavras-chave:** *Escherichia coli*, *Serratia liquefaciens*, multirresistência.

## 1. Introduction

It is likely that tens of millions of wild animals are illegally traded every year in Brazil, a situation that has serious implications for both conservation efforts and public health. It is impossible to inspect non-certified breeders to ensure the appropriate welfare and hygiene of their animals. As a result, uncertainties persist about appropriate management practices. Wild animal trafficking increases the risk of transmission of viruses, bacteria and zoonotic parasites from illegally traded pet animals, both within Brazil and beyond its borders <sup>(1)</sup>.

The vulnerability of birds to infections by bacterial pathogens of the order Enterobacterales, such as *Escherichia coli* and *Salmonella* spp., can increase due to stress caused by poor handling and unsanitary conditions <sup>(2,3)</sup>. Several studies involving both wild birds in the wild or in captivity, as well as domestic birds, have demonstrated that bird feces are a source of bacteria capable of compromising human and animal health. Avian feces can also transmit antimicrobial-resistant bacteria and resistance genes <sup>(4,5,6,7)</sup>.

Antimicrobial resistance is a significant concern in the global spheres of human and veterinary medicine <sup>(8)</sup>. Although this problem has been reported mainly in passerines raised in domestic environments and associated with the indiscriminate use of antibiotics <sup>(5,6)</sup>, wild birds can also contribute to spreading antimicrobial resistance via indirect exposure to antibiotics <sup>(8)</sup>. Some studies have also highlighted the problem of antimicrobial resistance in bacterial isolates important for human and animal health in birds rescued from animal trafficking and rehabilitated in specialized centers <sup>(3,9,10)</sup>. To ensure that wild birds that are released do not serve as possible sources of infection to other birds, it is important to carry out bacteriological monitoring <sup>(11)</sup>.

Wild birds remain rarely studied in this context, despite their relevance to pathogen transmission. Wild birds can also shed light on dietary and environmental influences on intestinal microbial structure and function. Despite the wide diversity of birds, studies of their intestinal microbiota are 10 times less numerous than studies devoted to mammals. Furthermore, bird studies are mainly conducted on domestic birds, especially those involved

in commercial meat production <sup>(12)</sup>. It is accordingly important to focus on understudied wild species involved in illegal trade and breeding. One example is passerines in the genus *Sporophila*. These birds are among the most affected by illegal trade and captive breeding, due to their small size, straightforward care and high singing capacity <sup>(13,14)</sup>. Here, we evaluate the occurrence and profile of antimicrobial resistance in enterobacteria isolated from passerines of the genus *Sporophila* originating from wild animal trafficking; our goal was to understand the implications of antimicrobial resistance on bird health and potential risks to human health.

## 2. Material and methods

We examined 35 wild passerines representing four different species of the genus *Sporophila* (*S. lineola*, n=27; *S. albogularis*, n=5; *S. nigricolis*, n=2; *S. caerulea*, n=1), in order to assess the presence of enterobacteria and analyze the birds' antimicrobial resistance profiles. The birds, originating from open-air markets and/or illegal breeding, were sent to the Ornithological Studies Laboratory (LABEO) of the Veterinary Faculty of the State University of Ceará from April–July 2022 by the Environmental Protection Department (DPMA). The aim of the LABEO was to assess the health of the birds and provide them with medical treatment, if necessary. Next, the birds were sent to the Instituto Pró-Silvestre (IPS), which was responsible for either releasing the birds or transporting them to legal breeders. The procedures were authorized by the Chico Mendes Institute for Biodiversity Conservation (SISBIO, registration 71437-4) and by the Ethics Committee for Animal Use of the State University of Ceará (27062020/2020).

Before collecting any material for microbiological analysis, the birds were evaluated for signs of illness. None of the animals appeared sick. We collected cloacal swabs and incubated the samples in 10 mL of 1% buffered peptone water at a temperature of 37°C for 24 h. Next, we inoculated 1 mL of the culture in 10 mL of cystine selenite (SC), Brain Heart Infusion (BHI) and Rappaport-Vassiliadis broths. The solutions were subsequently plated on MacConkey agar, brilliant green agar and *Salmonella-Shigella* agar simultaneously for selective growth of Enterobacteriaceae. Colonies of different morphologies were selected on each plate and subjected to the following biochemical tests: TSI agar (triple sugar-iron), SIM (motility and indole), LIA (lysine decarboxylase), ornithine decarboxylase, methyl red, urea, citrate Simmons, malonate, Voges-Proskauer, production of H<sub>2</sub>S and fermentation of glucose (with gas production), lactose, sucrose, mannitol, arabinose, raffinose, dulcitol, adonitol, inositol and sorbitol<sup>(15)</sup>.

Following the recommendations of the Clinical and Laboratory Standards Institute <sup>(16)</sup>, the isolates were subjected to the Kirby-Bauer disc diffusion technique. Antimicrobial discs were arranged on a plate containing Mueller-Hinton agar that had been previously seeded with the bacterial sample. The inhibition halos were measured after incubation at 37°C for 24 h. The *E. coli* ATCC 25922 strain was used as a control. To evaluate bacterial sensitivity, we tested 12 antibiotics belonging to 10 different pharmacological classes: aminoglycosides (gentamicin, 10 µg and tobramycin, 10 µg), penicillins in combination with beta-lactamase

inhibitors (amoxicillin with clavulanate, 30 µg), cephalosporins (ceftriaxone, 30 µg), amphenicols (chloramphenicol, 30 µg), fluoroquinolones (enrofloxacin, 5 µg, ciprofloxacin, 5 µg), folate-pathway inhibitors (sulfamethoxazole-trimethoprim, 1.25–23.75 µg), tetracyclines (tetracycline, 30 µg), carbapenems (meropenem, 10 µg), monobactams (aztreonam, 30 µg) and phosphonic acid (fosfomicin, 200 µg).

Samples in which there was no formation of a halo or when the halo was formed incompletely were considered to be resistant (R). Isolates were classified as multi-drug resistant when they demonstrated resistance to at least three classes of antibiotics <sup>(17)</sup>. Cases of intrinsic resistance were disregarded when we calculated our results.

3. Results

Twenty-three samples (65.7%) were positive for at least one bacterium in the order Enterobacterales. The most commonly isolated bacteria were *E. coli* (10 samples; 28.6%) and *S. liquefaciens* (9 samples; 25.7%). Another seven bacteria were isolated as well: *Proteus mirabilis* (11.4%), *Pantoea agglomerans* (11.4%), *Klebsiella* spp. (5.7%), *Citrobacter freundii* (2.9%), *Enterobacter cloacae* (2.9%), *Salmonella* spp. (2.9%), *Serratia rubidaea* (2.9%) and *Shigella* sp. (2.9%) (Table 1).

Of the 33 bacterial isolates that we analyzed, one of them was lost during processing. We found that ciprofloxacin was associated with the highest resistance rate (28.1%), followed by tetracycline (25.0 of 28 isolates) and enrofloxacin (18.2% of 32 isolates). The highest rates of resistance were observed for *S. liquefaciens*: 66.6% of samples were resistant to ciprofloxacin and 33.3% were resistant to enrofloxacin. Twenty percent of the *E. coli* samples were resistant to tetracycline and sulfazotrim, and two of the 10 isolates evaluated were not susceptible to these antibiotics (Table 2).

**Table 1 Absolute and relative frequencies of enterobacteria isolated from cloacal samples of birds in the genus *Sporophila* seized and sent to the Ornithological Studies Laboratory/FAVET/UECE in 2022**

Bacterium	N	%
<i>Escherichia coli</i>	10	28.6
<i>Serratia liquefaciens</i>	9	25.7
<i>Proteus mirabilis</i>	4	11.4
<i>Pantoea agglomerans</i>	4	11.4
<i>Klebsiella</i> spp.	2	5.7
<i>Citrobacter freundii</i>	1	2.9
<i>Salmonella</i> spp.	1	2.9
<i>Serratia rubidaea</i>	1	2.9
<i>Shigella</i> sp.	1	2.9
Samples positive for enterobacteria	23	65.7%

Out of the 32 resistant samples, we observed that 23 (71.9%) demonstrated antimicrobial resistance to at least one of the classes of antibiotics we tested. The highest incidence of resistant bacterial cultures (37.5%) when considering a single specific class of antibiotics, which corresponds to 12 isolates. Multi-drug resistance were observed in six isolates (18.8%), five of which (15.6%) were resistant to three classes of antibiotics. One case (3.1%) was resistant to eight classes of antibiotics. No cases of multi-drug resistance were observed in *Escherichia coli* isolates, five (50.0%) exhibited resistance to at least one antibiotic, and three specimens (30.0%) exhibited resistance to two antibiotics (Table 3).

**Table 2 Absolute and relative frequencies of antimicrobial resistance\* of enterobacteria from cloacal swabs of birds in the genus *Sporophilla***

Antibiotic	Bacteria			
	<i>Escherichia coli</i> (n=10)	<i>Serratia liquefaciens</i> (n=9)	Other enterobacteria (n=13)	Total (n=32)
AMC	1/10 (10.0%)	RN*	2/8* (25.0%)	3/18* (16.6%)
ATM	-	1/9 (11.1%)	1/13 (7.7%)	2/32 (6.3%)
CEF	-	2/9 (22.2%)	1/13 (7.7%)	3/32 (9.4%)
CIP	1/10 (10.0%)	6/9 (66.6%)	2/13 (15.4%)	9/32 (28.1%)
CLO	-	1/9 (11.1%)	2/13 (15.4%)	3/32 (9.4%)
ENR	1/10 (10.0%)	3/9 (33.3%)	2/13 (15.4%)	6/32 (18.8%)
FOS	-	2/9 (22.2%)	2/13 (15.4%)	4/32 (12.5%)
GEN	1/10 (10.0%)	-	-	1/32 (3.1%)
MER	-	-	2/13 (15.4%)	2/32 (6.3%)
TET	2/20 (20.0%)	2/9 (22.2%)	3/9* (28.6%)	7/28* (25.0%)
SUT	2/20 (20.0%)	1/9 (11.1%)	-	3/32 (9.4%)
TOB	-	-	-	-

RN-Natural resistance; AMC-Amoxicillin + Clavulanic acid; ATM-Aztreonam; CEF-Ceftriaxone; CIP-Ciprofloxacin; CLO-Chloramphenicol; ENR-Enrofloxacin; FOS-Phosfomycin; GEN-Gentamicin; MER-Meropenem; TET-Tetracycline; SUT-Sulfazotrim; TOB-Tobramycin \*Ignoring natural resistance.

**Table 3 Absolute and relative frequencies of multi-drug resistance of enterobacteria from cloacal swabs of birds in the genus *Sporophilla***

Number of classes of antibiotics	Number of total resistant isolates (%)	Number of resistant <i>E. coli</i> isolates (%)
0	9 (28.1%)	5 (50.0%)
1	12 (37.5%)	2 (20.0%)
2	6 (18.8%)	3 (30.0%)
3	5 (15.6%)	-

4. Discussion

The clinical significance of enterobacteria in Passeriformes is controversial given varied interpretations of these bacteria in the intestinal microbiota. According to Dorrestein<sup>(18)</sup>, *E. coli* and other enterobacteria do not occur in the intestines of healthy passerines; their presence is therefore associated with sick birds. However, other researchers have clarified that the

presence of *E. coli* in intestinal samples of certain specific types of birds, such as granivores, represents a health concern <sup>(19,20)</sup>. However, several investigations have also reported the presence of enterobacteria in granivorous passerines raised in the wild or in cages; like the birds that we studied, those animals did not present any signs of illness <sup>(5,6,21)</sup>.

We note that it is difficult to compare the rate of enterobacteria-positive samples (65.7%) with the findings of other studies of passerines, especially wild ones. That situation derives from the relative lack of comparable studies (i.e., investigations that used same sampling conditions). Furthermore, studies often differ in methodological differences (e.g., in relation to the species investigated or the microbiological procedures adopted). Most analyses of the total enterobacteria in the cloacas of wild bird have been limited to a single taxonomic order: parrots <sup>(10,22-25)</sup>. However, most research on parrots, has focused on analyzing smaller quantities of bacterial species, using either cloacal swabs or fecal samples collected in the environment. That approach differs from the one we adopted here. Generally, other studies have targeted *Salmonella* or *E. coli* <sup>(26-31)</sup>. Investigations aimed at detecting a more restricted set of microorganisms tend to more precisely characterize those microorganisms. That situation, in turn, enables a more in-depth analysis of the samples.

Horn et al. <sup>(5)</sup> used a microbiological methodology similar to that of this research with Belgian canaries (*Serinus canaria*) raised in cages in a domestic environment. These results, in addition to focusing on granivorous birds of the passerine order, also collected cloacal swabs and detected a lower total enterobacteria isolation rate (i.e., 10.9%) than we did (i.e., 65.7%). In another study involving Belgian canaries, but in this case focusing on fecal samples from collected from the bottom of the animals' cages, Beleza et al. <sup>(6)</sup> noted a yet a different total enterobacteria isolation rate (i.e., 54.5%). It is important to highlight that fecal collection, due to the greater volume of material available for microbiological processing compared with cloacal swabbing, presents a greater bacterial load. That situation can result in a higher rate of isolation of microorganisms <sup>(10,32)</sup>. However, even with cloacal swabs the higher rate of positivity of enterobacteria that we obtained can be explained by the stress imposed on the birds we studied due to inadequate nutrition and poor management conditions. That stress in turn likely triggered imbalances in the birds' intestinal microflora and promoted the proliferation of Enterobacteriaceae <sup>(33,34)</sup>. This situation is often observed in birds involved in illegal trafficking. Another factor that must be taken into consideration, regardless of the condition of animals in captivity, is the migratory habits of the genus *Sporophila*. Those habits might predispose these birds to infections by pathogenic microorganisms. Hubálek <sup>(35)</sup> noted that migratory behavior generates stress in birds, which can influence the excretion of pathogenic agents.

Passerines that have been trafficked have been shown to exhibit even higher rates of isolation of enterobacteria than we found. Matias et al. <sup>(36)</sup> analyzed passerines from different families at a wild animal rehabilitation center. Sixteen of the team's samples were from birds in the Thraupidae family, which also encompasses the genus *Sporophila*. Matias et al. observed that all of their samples were positive for enterobacteria. However, it is pertinent to highlight that the birds that Matias et al. analyzed were omnivorous. Their diet may have accordingly



influenced their intestinal bacterial; diet can play a significant role in differences in the gut microbiomes of certain bird species. For example, Gram-negative bacteria naturally make up a significant part of the gut microbiome of insectivorous birds, such as barn swallows (*Hirundo rustica*), as well as omnivorous species <sup>(22,37)</sup>. Therefore, the presence of enterobacteria is not a universal characteristic of birds that are omnivorous, insectivorous or granivorous. The frequency of detected enterobacteria can additionally vary between different species and individuals and is subject to a variety of influences.

Our microbiological analysis of cloacal samples from birds studied revealed the general presence of commensal bacterial species normally associated with passerines. These bacterial species can affect the animals' health in circumstances such as opportunistic infections <sup>(2)</sup>. *Escherichia coli* was the bacterium most frequently isolated in our study and was noted in 28.6% of the samples. However, we recovered considerably lower isolation rates compared with a study that investigated several species of illegally traded omnivorous passerines from the Thraupidae Family<sup>(36)</sup> and a study that analyzed only illegally traded cardinals (*Paroaria coronata* and *Paroaria dominicana*) <sup>(2)</sup>. Both of those studies identified rates above 85%. However, lower isolation rates (i.e., 10.7%) have also been observed by Braconaro et al. <sup>(9)</sup>; that team studied cloacal swabs from seized passerines. However, it is important to consider that using fewer biochemical tests to identify enterobacteria may influence the results obtained. *Serratia liquefaciens* was our second most commonly isolated bacterium (25.7%). Although Cunha et al. <sup>(2)</sup> detected *S. liquefaciens* in 18.3% of samples from cardinals (*Paroaria coronata* and *Paroaria dominicana*), other investigations of both domestic and wild specimens in the order Passeriformes generally recovered considerably lower rates <sup>(2,5,6,9,36,38)</sup>.

Among the bacteria that we isolated, the most important in terms of both animal and human health is *E. coli*. Although *E. coli* is known to be a commensal microorganism, these bacteria become problematic in secondary infections or when their genes are involved in specific mechanisms of virulence. Such situations can cause a variety of complications in humans, including intestinal and urinary disorders <sup>(28)</sup>. Strains known as Avian Pathogenic *E. coli* (APEC) have been noted to be responsible for colibacillosis in the poultry industry worldwide; however, in wild birds, information about APEC is very limited, but natural infections and cases of the disease have been described <sup>(39)</sup>. Members of the genus *Serratia* are recognized to be a cause of opportunistic infections in humans; however, such infections can also occur in animals and insects, particularly the species *S. marcescens* <sup>(40)</sup>. Despite the paucity of studies, *S. liquefaciens* has been shown to be the second most frequently documented species in the genus *Serratia* in clinical laboratories associated with infections in humans, especially those of nosocomial origin <sup>(41)</sup>. A case of pyoderma gangrenosum possibly caused by this microorganism was reported in a 59-year-old diabetic patient who was attacked by a crow in Spain <sup>(42)</sup>. Although this species is potentially pathogenic to songbirds and can cause systemic infections <sup>(38)</sup>, the occurrence of disease is uncommon. However, disease can occur in immunocompromised hosts <sup>(43)</sup>.

Although other isolated bacteria have been reported less frequently, it is still important to consider such species due to their clinical relevance and potential impacts on human and

animal health. For example, bacteria from the genera *Salmonella*, *Shigella* and *Proteus* are recognized to be significant zoonotic pathogens; their occurrence in animals may represent a threat to humans <sup>(44)</sup>. Therefore, even if these bacteria occurred in an asymptomatic bird, their presence must be viewed with caution, as it does not rule out the possibility of being pathogenic for both humans and other animals. Belez et al. <sup>(6)</sup> demonstrated the presence of *Salmonella* spp. in asymptomatic, domestic Passeriformes. However, it is important to highlight that pathogenicity is directly linked to a specific serotype capable of causing illness in certain species of birds. The *Salmonella* Typhimurium serotype, for example, has been reported to be an important cause of disease in parrots, *Salmonella* Gallinarum has been associated with pathogenic manifestations in quails and *Salmonella* Enteritidis has been linked to outbreaks in humans <sup>(28,32,45)</sup>. We were unable to perform serotyping in this study.

Upper respiratory tract infections and deep pododermatitis related to bacteria in the genus *Proteus* have been reported in wild birds <sup>(46)</sup>. However, it is important to highlight that the most relevant bacterial species to birds is *Proteus mirabilis*. This pathogen, which widely distributed in nature and is part of the natural intestinal microbiota of animals, is considered to be opportunistic and is associated with human urinary tract infections. *Proteus mirabilis* is concerning to poultry industry because it can be potentially transmitted by chicken carcasses <sup>(15,47)</sup>. However, Marques et al. <sup>(48)</sup> noted that companion animals can also be possible sources of *Proteus mirabilis*; these authors found that a large number of *P. mirabilis* strains were common among companion animals and humans. *P. mirabilis* was detected in 17.7% of cloacal swabs from parrots housed in pet stores <sup>(49)</sup>. That finding demonstrates the importance of birds in this context. Reports of bacteria in the genus *Shigella* sp. in wild birds are scarce. To date, there have been no studies of the presence of this microorganism in passerines. Recently, however, *Shigella* sp. was found in several species of free-ranging wild and migratory waterfowl (e.g., *Anastomus oscitans*, *Anhinga melanogaster* and *Ciconia ciconia*) for the first time <sup>(50)</sup>. Previously, *Shigella* sp. had been found in raptors and herons in a zoo in Madagascar <sup>(51)</sup>. Severe diarrhea and bloody feces caused by *Shigella* sp. have been reported in chickens; the natural host is conventionally humans <sup>(52)</sup> in the form of shigellosis, which is one of the primary causes of diarrhea in countries such as India <sup>(53)</sup>.

*Citrobacter freundii* and *Klebsiella* sp. were two of the least frequently isolated bacteria in this research. These species have also been isolated at low frequencies in asymptomatic domestic or wild passerines in other studies <sup>(5,38)</sup>. On a previous occasion, they were reported to cause mortality linked to septicemia and pneumonia, respectively, in passerines confiscated and sent to a wild bird screening center in São Paulo, Brazil<sup>(54)</sup>. This finding highlights the importance of considering the pathogenicity of these agents and invoking preventive measures to ensure bird health. Such precautions are particularly critical given the wide ecological distribution of these pathogens and other members of the Enterobacterales order. These microorganisms occupy a variety of niches, both in the intestines of their vertebrate hosts and in extra-intestinal habitats, which reinforces the importance of understanding their potential impact on bird health<sup>(55)</sup>.



Some of the bacteria isolated in this research, such as *Pantoea agglomerans*, are generally recognized to be non-pathogenic to humans and animals. The presence of *Pantoea agglomerans* in intestinal microbiota of birds can be explained by the animals coming into contact with seeds and their surrounding environment. Studies have reported the presence of these agents in fecal samples from healthy passerines, as well as from other orders of birds, living both in captivity and in the wild <sup>(5,6,22,49)</sup>. Despite occurring in vertebrates and invertebrates, the clinical importance of *Pantoea agglomerans* in animals remains poorly documented. In relation to birds, there are few scientific references on infections caused by this bacteria. Some beneficial effects of this bacterium are known—for instance, it produces antibiotics potentially important for combating plant, animal and human pathogens. *Pantoea agglomerans* also aids as in food preservation <sup>(56)</sup>. Furthermore, it has been observed that *P. agglomerans* is associated with improved digestibility of fruit seeds in birds <sup>(57)</sup>. Despite being considered harmless, it is important to highlight that this bacterial species carries out important exchanges of genetic materials that determine pathogenicity during host colonization with other members of the Enterobacteriaceae family, including species pathogenic to humans <sup>(58)</sup>. This microorganism can also cause occupational diseases of allergic and/or immunotoxic origin, as well as accidental infections in humans<sup>(56)</sup>. Reports of *P. agglomerans* infections in humans are rare, but one example was the report of an unusual case of cervical spondylodiscitis resulting from trauma. This organism is generally associated with penetrating trauma from contaminated plant material or intravenous products <sup>(59)</sup>.

Our data regarding antimicrobial resistance are both similar and different from other investigations involving domestic or wild passerines living in both the wild and captivity. This variation can be mainly attributed to discrepancies in the birds' level of exposure to antibiotics, disparities in the methodologies used and variations in the types of antibiotics investigated. When considering the total population of bacteria, the resistance rates in the isolates in our research were far higher for ciprofloxacin (28.1%), tetracycline (25.0%) and enrofloxacin (18.8%). Lopes et al. <sup>(10)</sup> analyzed isolates from the cloacal swabs of parrots taken to a wild animal screening center and tested some of the same antibiotics used in our research. Lopes et al. observed that bacteria evaluated with tetracycline exhibited a higher percentage of resistance (37.3%); enrofloxacin (9.3%) and ciprofloxacin (5.6%) exhibited lower percentages. These authors also obtained low resistance rates for gentamicin (2.5%); however, they recorded comparatively higher percentages for sulfazotrim (28.6%). Horn et al. <sup>(5)</sup> studied Belgian canaries raised in cages and noted resistance rates (55.7%) that surpassed those of our study (ciprofloxacin). However, it is important to note that the disc used by these researchers (sulfonamides, 300 µg) was not tested in our investigation. The resistance rates for tetracycline and sulfazotrim were also higher (39.3% and 29.5%, respectively); the resistance rate for enrofloxacin was 6.6%. In free-ranging birds, resistance rates for gentamicin (18.2%), ceftriaxone (20.0%) and meropenem (14.5%) were higher than those detected in our investigation; the opposite situation held true for tetracycline (9.1%) and ciprofloxacin (9.1%) <sup>(22)</sup>.

In terms of the percentages of resistance per isolate, we observed the two highest rates for the bacterium *Serratia liquefaciens*. The highest was noted when ciprofloxacin was used

(66.6%), and the second highest was noted when enrofloxacin was applied (33.3%). Gaio et al. <sup>(3)</sup> analyzed samples of cloacal swabs from illegally traded passerines and detected higher resistance rates. Compared with *E. coli*, they obtaining 91.8% for ciprofloxacin and 77.8% for enrofloxacin. Braconaro et al. <sup>(9)</sup> also examined the presence of *E. coli* in passerines confiscated and sent for rehabilitation. They observed high percentages of resistance in relation to amoxicillin + clavulanic acid (100.0%). However, for tetracycline (22.2%) and enrofloxacin (11.1%), the results were closer to those detected in our research.

When we assessed multi-drug resistance of total enterobacteria, we obtained similar results to studies that used a similar methodology and investigated birds in a natural environment. Those studies also disregarded intrinsic resistance rates, which can result in overestimates. Those studies reported resistance rates both below (i.e., 11.1%) and slightly higher (i.e., 23.4%) than those found in our research <sup>(21,60)</sup>. In birds kept in cages in domestic farms, Beleza et al. <sup>(6)</sup> were able to identify higher rates of multi-drug resistance; those authors reported up to 37.7% of total canary Enterobacteriaceae isolates. Marques et al. <sup>(49)</sup> investigated parrots intended for domestic breeding housed in pet stores and observed a higher rate of multi-drug resistance. However, their rate was much closer to that found in our research (i.e., 18.0%). We did not identify any cases of multi-drug resistance in *E. coli*. Only two classes of antibiotics affected at most one isolate, and those antibiotics were effective in 30.0% of cases. However, the literature reveals high rates of antimicrobial resistance to this bacterial species. For example, 67.0% of *E. coli* samples from pet birds (passerines and psittaciforms) in Istanbul were multi-drug resistant <sup>(61)</sup>. Furthermore, even higher rates were obtained by Braconaro et al. <sup>(9)</sup>, who reported that 92.6% of isolates from passerines living in a rehabilitation center were multi-drug resistant.

It is not possible to determine the life history of the passerines that we analyzed (i.e., whether or not they had been kept for a long time in illegal facilities). However, it is reasonable to assume that the lower rate of occurrence of antimicrobial resistance that we observed can be attributed to the birds' reduced exposure to antibiotics and limited contact with resistant bacteria during their stay in enclosures or even before their capture. And if we consider that some or all of the passerines had recently been captured in the wild, their natural food-seeking behavior may have also influenced the resistance rates that we observed. Although passerines in the genus *Sporophila* have migratory characteristics and come into contact with different environments, they have predominantly granivorous eating habits—they move preferentially to areas with an abundant and diverse supply of seeds, and they land on plant stems to feed on seeds <sup>(62,63)</sup>. Other bird species that exhibit this behavior have also been reported to be less likely to harbor microorganisms resistant to antimicrobials compared with omnivorous species with anthropogenic habits <sup>(64)</sup>.

Some of the specific cases of antibiotic resistance that we observed deserve special attention; one such case is meropenem (6.3%). This antibiotic is used in hospitals <sup>(21)</sup>. In general, all cases of antimicrobial resistance detected in this study, including multi-drug resistance, must be considered relevant given the prospect of reintroducing these birds to the natural environment. There is ample evidence suggesting that free-ranging wild birds have

the ability to migrate long distances over short periods of time. Such migration can result in the transport of resistant bacteria, which, in turn, have the potential to be transmitted non-invasively to humans and also other wild or domestic animals <sup>(4,21)</sup>. Therefore, it is important that environmental agencies consider the problem of multi-resistance to antimicrobials before releasing wild birds. Otherwise, the spread of these agents throughout the environment, via contaminated feces, could become a true threat <sup>(10)</sup>.

## 5. Conclusion

Birds in the genus *Sporophila* that we analyzed at the Laboratory of Ornithological Studies of the State University of Ceará exhibited several members of the order Enterobacterales; *E. coli* and *S. liquefaciens* were the most common. The resistance rates of the antibiotics that we investigated were not high. Considering each bacterium individually, the resistance results that were most significant were noted for *S. liquefaciens*. There was a low frequency of antimicrobial multi-resistance in total isolates; such multi-resistance was not observed in *E. coli* samples.

## Declaration of conflict of interest

The authors declare no conflicts of interest.

## Author contributions

Conceptualization: Melo LS, Paschoal Filho NM, Beleza AJF. Formal analysis: Teixeira RSC, Paschoal Filho NM, Silva ING. Research, Methodology: Freitas CMP, Melo LS, Ribeiro CDS, Fontão CC. Resources: Lima BP, Silva ING. Validation: Maciel WC. Project management: Melo LS, Teixeira RSC. Supervision: Melo LS, Teixeira RSC. Writing (original draft): Teixeira RSC. Writing (later drafts): Marques AR, Lopes ES (review and editing): Teixeira RSC.

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