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Frequency of zoonotic bacteria among illegally traded wild birds in Rio de Janeiro

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ABSTRACT

The illegal wildlife trade may increase the risk of infectious disease transmission, and it may not only cause disease outbreaks in humans but also threaten livestock, native wild populations, and ecosystems' health. Bird species may act as carriers in the transmission of enteric pathogens. However, epidemiological studies on zoonotic bacteria in wild birds are rare in Brazil. From March 2011 to March 2012, we investigated the frequency of Enterobacteriaceae in cloacal swab samples from 109 birds of the passerine and Psittacidae families. These birds were recovered from illegal trade in Rio de Janeiro, Brazil, and sent to a rehabilitation center. Gram-negative bacteria were isolated from 86 wild birds (78.9%). A mean (±SD) of 1.68 (±1.30) different bacterial species were isolated per bird, with a maximum of five bacterial species from three bird species. The most frequently isolated bacteria were *Escherichia coli*, followed by *Enterobacter* spp., *Klebsiella pneumoniae* and other enteric bacteria. *Salmonella* ser. Typhimurium was isolated from a Temminck's seedeater (*Sporophila falcirostris*), and two *Salmonella* ser. Panama were isolated from two specimens of chestnut-capped blackbird (*Chrysomus ruficapillus*). Of the 70 selected bacterial isolates, 60 exhibited antibiotic resistance. The resistance patterns varied from one to nine of the antibiotics tested. Resistance to ceftiofur was the most prevalent, followed by ampicillin and ceftriaxone. The dissemination potential of resistant strains in situations typically seen in the management of captive birds may become a problem for the conservation of natural bird populations and for public health.

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Introduction

The illegal wildlife trade is considered the most lucrative illegal activity in the world, after weapons and illicit drug commerce.[1–4] According to the Brazilian laws, capturing wild animals and maintaining them in captivity without a legal permit is a crime. Because Brazil is one of the richest countries in the world in terms of biodiversity,[5] birds are captured both for national and international trade. When confiscated by official authorities, these birds are sent to rehabilitation centers.[4,6]

After habitat loss, the poaching and hunting of wildlife are considered the most important causes of population declines and could significantly affect an ecosystem's dynamics.[7] In addition to these consequences, the risk of disease transmission has to be considered given that captivity allows a more intense contact among species, which favors the transmission of infectious agents.[8–10] Moreover, captive practices enable disease transmission mechanisms that not only can cause outbreaks in humans but also threaten livestock, native wildlife populations, and affect ecosystems' health.[11]

Wild birds and migratory species may act as sources of infections in the transmission of different microorganisms and may play a role in the spreading of emerging and re-emerging pathogens.[12–14] These birds are susceptible to various bacterial pathogens common to men and domestic animals in addition to other potential pathogenic microorganisms, such as protozoa and viruses.[14,15]

Studies on the microbiota of wild birds are rare or limited to a small number of animals, and those addressing the prevalence of Enterobacteriaceae are especially focused on certain groups, such as seagulls. More specifically, research on passerines covered outbreaks with high mortality, which provides no information on the prevalence of pathogens in apparently healthy animals. Thus, the role of these birds as reservoirs of bacterial pathogens may indeed be underestimated.[14]

Zoonotic gram-negative bacteria previously isolated from both apparently healthy and sick avian hosts included *Salmonella* spp., *Escherichia coli*, *Campylobacter* spp., *Yersinia* spp., *Klebsiella* spp. and *Enterobacter* spp. Except for the last two etiologic agents, which do not cause disease under normal conditions, these bacteria are responsible for gastroenteritis, respiratory symptoms, septicemia, and even mortality in humans.[14–16]

The use of antibiotics in animals to control bacterial infections or as growth promoters in poultry production may result in the selection of resistant strains of pathogenic bacteria as much as those that form the normal microbiota. These practices are considered the main factor for triggering the emergence, selection and spread of resistant microorganisms, both in veterinary and human medicine. Although species do not have contact with antibiotics in the wild, they can be infected by wild birds that act as carriers given that antibiotic-resistant bacteria have been isolated in these animals. In addition to the potential problem for wildlife conservation, the spread of multi-drug resistant strains may have implications for public health. The manipulation of these animals and the disposal of their waste represent a hazard for the professionals involved in the surveillance/policing activities, such as veterinarians, biologists, and caregivers.[14–16]

To better assess the risk of exposure to zoonotic bacteria carried by wild birds for these professionals, we conducted a prevalence survey in a rehabilitation center to describe and compare the frequency of Enterobacteriaceae among groups of birds. The potential pathogenicity to humans was analyzed by the presence of toxin genes in selected isolates of *E. coli*. Furthermore, we tested the antibiotic resistance in selected strains that were representative of the isolated bacterial species.

Materials and methods

Wild bird specimens where sampled upon arrival at the Rehabilitation Center of Wild Animals (CETAS) in Seropédica, Rio de Janeiro State, Brazil, after being confiscated from local illegal trade markets by the authorities from March 2011 to March 2012. The scientific nomenclature of the bird species follows the Brazilian Ornithological Records Committee (CBRO). Cloacal samples were obtained from one hundred and nine birds of 30 species that were randomly chosen in a total of nine apprehensions. The samples were taken following clinical procedures. Swabs were introduced in Cary Blair medium under refrigerated conditions and sent to the Enterobacteria Laboratory of the Oswaldo Cruz Institute (FIOCRUZ), in Rio de Janeiro, Brazil for microbiological assays. All the procedures were approved by the Chico Mendes Institute of Biodiversity Conservation (SISBIO no 26383-2) and by the Fiocruz Ethics Committee on the Use of Animals (LW – 1/13).

The collected material was transferred to a nutrient broth (Difco™; 37 °C/18–24 h). Then, the samples were enriched in a Rappaport–Vassiliadis broth (42 °C overnight), a Silliker medium and a Muller–Kauffmann medium (37 °C/18–24 h). Next, the cultures were plated for isolation on Hektoen enteric agar (Oxoid™; 37 °C/18–24 h). Representatives of all the distinct colonies were confirmed in a triple sugar iron test (Difco™) and inoculated into a SIM medium for the biochemical characterization of several parameters such as the susceptibility to L-lysine decarboxylase, citrate as a carbon source, mobility, hydrogen sulfide production, glucose and lactose fermentation as well as the indole production. The presumptive diagnosis of the distinct gram-negative isolates was performed by the biochemical tests recommended by Murray et al.[17] and Murray et al.[18].

The subspecies of *Salmonella* spp. were determined using substrates according to Grimont and Weill.[19] The antigenic characterization, which included an induction/absorption phase to recognize the somatic and flagellar fraction, was performed by slide agglutination with somatic and flagellar poly- and monovalent antigens based on the Kaufmann–White scheme.

To compare the frequencies of bacteria isolated from groups of birds, Fisher's exact test was performed using the SPSS software package. A two-way general linear model analysis of variance (ANOVA) was used to examine the differences in species richness of bacteria isolated from different bird families and from the most common bird species. p values of 0.05 or less were considered significant. Species richness values were square-root transformed for normality.

A susceptibility test was performed with 70 isolates of *E. coli*, *Klebsiella* spp. and *Salmonella* spp., from 54 birds using the minimum inhibitory concentration assay (MIC) in agar and broth to determine the lowest concentrations of different antimicrobial drugs. Each one was evaluated in a serial dilution according to the protocol described by the Clinical and Laboratory Standards Institute (CLSI)[20] with ampicillin, ceftriaxone, ceftiofur, tetracycline, sulfamethoxazole/trimethoprim 19:1, chloramphenicol, gentamicin, nalidixic acid, ciprofloxacin, enrofloxacin, and nitrofurantoin. The following reference strains were used for the quality control of the antimicrobial susceptibility test: *Staphylococcus aureus* ATCC25923, *Pseudomonas aeruginosa* ATCC27853, *Enterococcus faecalis* ATCC29212 and *E. coli* ATCC25922.

E. coli strains were selected to identify the presence of toxin genes with the multiplex PCR protocols established by Almeida et al.[21] used for the primary screening of enteropathogens in the Enterobacteria Laboratory of the Oswaldo Cruz Institute (FIOCRUZ), Rio de Janeiro, Brazil. The following genes were investigated: *eaeA*, *stx1*, *stx2*, *LT*, *ST*, *eagg* and *ipaH*.

Results

The most common sampled bird species were passerines that belonged to the families Emberizidae and Thraupidae (Table 1). Gram-negative bacteria were isolated from 86 of the 109 wild birds sampled (78.9%). A mean (±SD) of 1.68 (±1.30) different bacterial species were isolated per bird, with a maximum of five bacteria from three distinct bird species: a rufous-collared Sparrow (*Zonotrichia capensis*), a tsayaca Tanager (*Tangara sayaca*), and a green-winged saltator (*Saltator similis*). The most frequent isolated bacteria were *E. coli*, which were prevalent in 55 animals. The next most often isolated bacteria were, in decreasing order, *Enterobacter* spp. and *Klebsiella pneumoniae* (Table 2). *Salmonella* ser. Typhimurium was isolated from a Temminck's seedeater (*Sporophila falcirostris*), and two *Salmonella* ser. Panama were isolated from two specimens of chestnut-capped blackbird (*Chrysomus ruficapillus*) that were kept together in the same cage.

There were no significant differences in the frequencies of microorganism among the most common bird species, i.e., saffron finch ($n = 15$), blue-black grassquit ($n = 11$), and double-collared seedeater ($n = 11$), according to Fisher's exact test ($p < 0.05$). Likewise, based on Fisher's exact test ($p < 0.05$), species of Enterobacteriaceae were significantly more frequent in birds of the families Thraupidae (100%; $n = 16$), Cardinalidae (100%; $n = 8$), Turdidae (100%; $n = 6$), and Psittacidae (100%; $n = 3$) compared to birds of the families Icteridae (91.7%; $n = 12$) and Emberizidae (63.3%; $n = 60$; $p = 0.003$). The *E. coli* occurrence was significantly higher in birds of the family Psittacidae (100%; $n = 3$) than in birds of the families Thraupidae (87.5%; $n = 16$), Turdidae (83.3%; $n = 6$), Cardinalidae (62.5%; $n = 8$), Icteridae (58.3%; $n = 12$), and Emberizidae (28.3%; $n = 60$; $p < 0.05$). The frequency of *K. pneumoniae* was significantly higher in birds of the family Turdidae (66.7%; $n = 6$) compared to birds of the families Thraupidae (56.3%; $n = 16$), Icteridae (50%; $n = 12$), Cardinalidae (50%; $n = 8$), and Emberizidae (21.7%; $n = 60$: $p = 0.01$).

Table 1 – Wild birds sampled in the CETAS, Rio de Janeiro (Brazil) from March 2011 to March 2012. The total number of individual bird samples are shown.

Family	Species	Total
Emberizidae	Saffron finch (*Sicalis flaveola*)	15
	Blue-black grassquit (*Volatinia jacarina*)	11
	Double-collared seedeater (*Sporophila caerulescens*)	11
	Seedeater (*Sporophila* spp.)	8
	Buffy-fronted seedeater (*Sporophila frontalis*)	3
	Rufous-collared sparrow (*Zonotrichia capensis*)	5
	Temminck's seedeater (*Sporophila falcirostris*)	3
	Chestnut-bellied seed-finch (*Sporophila angolensis*)	2
	Lined seedeater (*Sporophila lineola*)	1
	Pileated finch (*Coryphospingus pileatus*)	1
Thraupidae	Sayaca tanager (*Tangara sayaca*)	5
	Golden-chevroned tanager (*Tangara ornata*)	4
	Brazilian tanager (*Ramphocelus bresilius*)	3
	Red-cowled cardinal (*Paroaria dominicana*)	3
	Ruby-crowned tanager (*Tachyphonus coronatus*)	1
Cardinalidae	Green-winged saltator (*Saltator similis*)	6
	Buff-throated saltator (*Saltator maximus*)	1
	Ultramarine grosbeak (*Cyanoloxia brissonii*)	1
Icteridae	Chestnut-capped blackbird (*Chrysomus ruficapillus*)	8
	Shiny cowbird (*Molothrus bonariensis*)	1
	Chopi blackbird (*Gnorimopsar chopi*)	3
Turdidae	Rufous-bellied thrush (*Turdus rufiventris*)	4
	White-necked thrush (*Turdus albicollis*)	1
	Creamy-bellied thrush (*Turdus amaurochalinus*)	1
Psittacidae	Maroon-bellied parakeet (*Pyrrhura frontalis*)	1
	White-eyed parakeet (*Aratinga leucophthalma*)	1
	Blue-fronted parrot (*Amazona aestiva*)	1
Fringillidae	Purple-throated euphonia (*Euphonia chlorotica*)	2
Estrildidae	Common waxbill (*Estrilda astril*)	1
Tyrannidae	Great kiskadee (*Pitangus sulphuratus*)	1
Total		109

The Enterobacteriaceae mean (±SD) species richness for each family was 1.1 (±1.06) in Emberizidae, 2.62 (±1.08) in Thraupidae, 2.16 (±1.46) in Icteridae, 2.62 (±1.40) in Cardinalidae, 2.33 (±1.03) in Turdidae, and 1.33 (±0.57) in Psittacidae. These differences were significant ($F = 6.71$, $p < 0.05$) based on a general linear model ANOVA (Tukey's post hoc test: Thraupidae > Emberizidae, $p < 0.05$; Cardinalidae > Emberizidae, $p = 0.01$). The mean bacterial species richness for the most common wild bird species was 1.26 (±0.96) for saffron finch, 0.81 (±0.98) for blue-black grassquit, and 0.81 (±0.75) for double-collared seedeater. These differences were not significant (DF = 1.08, $p = 0.35$) based on a general linear model ANOVA.

The *eaeA* gene was present in five of 61 *E. coli* isolates obtained from a white-necked thrush (*Turdus albicollis*), a chestnut-bellied seed-finch (*Sporophila angolensis*), a sayaca

Table 2 – Enterobacteriaceae from cloacal samples of wild birds in the CETAS, Rio de Janeiro, Brazil. The samples were collected from March 2011 to March 2012. The frequency of Enterobacteriaceae isolated from each bird family sampled are shown.

Bacteria isolated	Isolates from each bird family							
	Emberizidae $n=60$	Thraupidae $n=16$	Icteridae $n=12$	Cardinalidae $n=8$	Turdidae $n=6$	Psittacidae $n=3$	Misc. $n=4$	Total $n=109$
Escherichia coli	17 (28.3)	14 (87.5)	7 (58.3)	5 (62.5)	5 (83.3)	3 (100.0)	4 (100.0)	55 (50.5)
S Salmonella ser. Typhimurium	1 (1.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.9)
Salmonella ser. Panama	0 (0)	0 (0)	2 (16.7)	0 (0)	0 (0)	0 (0)	0 (0)	2 (1.8)
Citrobacter freundii	3 (5.0)	1 (6.3)	0 (0)	1 (12.5)	0 (0)	0 (0)	1 (25.0)	6 (5.5)
Other *Citrobacter*	1 (1.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.9)
Klebsiella pneumoniae	13 (21.7)	9 (56.3)	6 (50.0)	4 (50.0)	4 (66.7)	0 (0)	3 (75.0)	39 (35.8)
Klebsiella oxytoca	1 (1.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.9)
Enterobacter spp.	21 (35.0)	11 (68.8)	8 (66.7)	5 (62.5)	3 (50.0)	1 (33.3)	1 (25.0)	50 (45.9)
Enterobacter gergoviae	1 (1.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.9)
Enterobacter intermedius	0 (0)	0 (0)	1 (8.3)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.9)
Enterobacter aerogenes	0 (0)	0 (0)	0 (0)	0 (0)	1 (16.7)	0 (0)	0 (0)	1 (0.9)
Enterobacter cloacae	1 (1.7)	1 (6.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (1.8)
Hafnia alvei	1 (1.7)	0 (0)	0 (0)	3 (37.5)	0 (0)	0 (0)	0 (0)	4 (3.7)
Serratia spp.	4 (6.7)	2 (12.5)	0 (0)	2 (25.0)	0 (0)	0 (0)	0 (0)	8 (7.3)
Proteus spp.	0 (0)	1 (6.3)	1 (8.3)	0 (0)	0 (0)	0 (0)	0 (0)	2 (1.8)
Morganella morganii	0 (0)	2 (12.5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (1.8)
Providencia spp.	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (25.0)	1 (0.9)
Enterobacteriaceae	38 (63.3)	16 (100.0)	11 (91.7)	8 (100.0)	6 (100)	3 (100.0)	4 (100.0)	86 (78.9)

tanager (*T. sayaca*), a chestnut-capped blackbird (*C. ruficapillus*), and a chopi blackbird (*Gnorimopsar chopi*). The *stx2* gene was simultaneously present in the chopi blackbird sample.

Antibiotic resistance was present in 60 of the 70 selected bacterial isolates (Table 3). The resistance patterns varied from one to nine of the antibiotics tested. The resistance to ceftiofur (71.67%) was the most frequent, followed by ampicillin (46.67%) and ceftriaxone (35%).

Discussion

E. coli was the most frequently isolated bacteria from wild birds in this study, and its frequency was significantly higher in Psittacidae birds than in passerines. This microorganism is the most abundant facultative bacterial species in the normal microbiota of the large intestines of animals and humans,[22] and it has been isolated from a range of bird species, such as passerines.[14] However, its prevalence is higher in carnivorous or omnivorous bird species than in granivorous birds, such as most passerines in our study, which have a lower prevalence of this pathogen.[16] The higher frequency of *E. coli* in our study could be explained in light of the poor sanitary conditions under which the animals were maintained after being captured in the wild.

Enteropathogenic strains have been isolated from healthy or diseased wild birds, which may be carriers of *E. coli* strains resistant to antibiotics.[12] Of the five *E. coli* isolates that carried the *eaeA* gene, one carried simultaneously the *stx2* gene. The presence of both genes classifies the strain as an enteropathogenic (EPEC) or enterohemorrhagic (EHEC) *E. coli*.

The low frequency of *Salmonella* spp. isolated in this study is in agreement with previous studies with apparently healthy wild birds.[23,24] In spite of the low detection rate in the sampled animals and of the difficulty to collect biological material from birds seized in illegal wildlife trade, our results indicate that the isolated serovars circulate in natural bird populations. Several studies revealed that *Salmonella* ser. Typhimurium and *Salmonella* ser. Panama circulate in Brazil and in other countries and can be isolated from human and animal biological sources.[25–27]

Salmonella ser. Typhimurium is frequently associated with disease in several mammalian and avian host species, and it was shown to be common in outbreaks that affected humans and livestock, especially poultry, until the 1990s.[28,29] Outbreaks of *Salmonella* ser. Typhimurium infections in humans that had contact with wild passerine birds have been described in several European countries as well as in the U.S.A. and New Zealand.[30–32] Instead, serovar Panama is not frequently isolated in epizooties and human outbreaks in Brazil.[28]

Several of the isolated bacteria, such as *K. pneumoniae*, *Enterobacter* spp., *Proteus* spp., *Providencia* spp. and *Morganella morganii*, are known or suspected to cause diseases in humans and are often associated with nosocomial infections.[16]

Antibiotic resistance in gram-negative bacteria has been reported in other studies of wild birds.[16,33–37] Resistance to ampicillin is consistent with the results obtained by Steele et al.[16], Tsubokura et al.[33], Nascimento et al.[34], Silva-Hidalgo et al.[38], and Carroll et al.[37] The same is valid for the resistance to ceftiofur, as reported by Steele et al.[16]. Likewise, it is important to note the number of multi-drug resistant *E. coli* and *K. pneumoniae* strains, both of which are important nosocomial pathogens.[16]

These resistance profiles stress the importance of a surveillance program to prevent the impact of these pathogenic microorganisms on public health.[39] A considerable number of isolates and the three *Salmonella* strains were resistant

Table 3 – Enterobacteriaceae isolated from wild birds in the CETAS, Rio de Janeiro, Brazil, from March 2011 to March 2012. These bacteria were tested for antibiotic resistance.

Host species	Bacterial isolate	Antibiotic resistance											Total
		AMP	CRO	CEF	TCY	SXT	CHL	GEN	NAL	CIP	ENR	NIT	
Saltator similis	*Escherichia coli*	S	S	S	S	S	S	S	R	S	S	I	1
Sporophila angolensis	*Escherichia coli*	S	S	S	S	S	S	S	S	S	S	S	0
	Escherichia coli	R	R	R	S	S	S	R	S	S	I	S	4
	Klebsiella	R	I	R	R	R	R	S	R	R	R	R	9
Aratinga leucophthalma	*Escherichia coli*	S	S	S	R	S	S	S	S	S	S	S	1
Chrysomus ruficapillus	*Escherichia coli*	R	R	R	S	R	S	R	S	S	S	S	5
Turdus amaurochalinus	*Escherichia coli*	S	S	S	S	S	S	S	S	S	S	S	0
	Klebsiella	R	I	R	I	R	I	S	R	S	I	R	5
Amazona aestiva	*Escherichia coli*	R	R	R	S	S	S	I	S	R	S	R	5
Gnorimopsar chopi	*Escherichia coli*	I	R	R	S	R	S	R	R	R	R	I	7
	Escherichia coli	S	S	R	S	S	S	S	S	S	R	S	2
	Klebsiella	R	S	S	S	S	I	S	S	S	I	R	2
Tangara sayaca	*Escherichia coli*	R	S	S	R	S	S	S	S	S	S	R	3
	Escherichia coli	S	S	S	S	S	S	S	S	S	S	S	0
	Klebsiella	R	R	S	I	R	I	I	S	S	I	I	3
Euphonia chlorotica	*Escherichia coli*	R	1	R	S	S	S	S	S	S	S	R	3
	Klebsiella	R	S	I	S	R	I	S	S	S	I	R	3
Zonotrichia capensis	*Escherichia coli*	R	I	R	S	S	R	S	S	S	R	R	5
	Escherichia coli	S	S	S	R	S	S	S	S	S	S	R	2
Chrysomus ruficapillus	*Escherichia coli*	S	R	R	R	S	I	S	S	S	S	I	3
Chrysomus ruficapillus	*Escherichia coli*	S	S	R	S	S	S	S	S	S	I	S	1
	Klebsiella	R	S	S	S	S	S	S	S	S	I	R	2
Zonotrichia capensis	*Escherichia coli*	R	R	R	R	S	R	S	S	S	R	R	7
Euphonia chlorotica	*Escherichia coli*	R	S	R	R	R	R	S	R	S	I	R	7
Sporophila caerulescens	*Escherichia coli*	S	S	R	S	S	S	S	S	S	S	I	1
Sporophila spp.	*Escherichia coli*	S	S	S	S	S	S	S	S	S	S	S	0
	Escherichia coli	S	S	I	S	S	S	S	S	S	S	S	0
Pitangus sulphuratus	*Escherichia coli*	S	S	S	S	S	S	S	S	S	S	S	0
Pyrrhura frontalis	*Escherichia coli*	S	S	S	S	S	S	I	S	S	S	S	0
Ramphocelus bresilius	*Escherichia coli*	S	S	R	S	R	S	I	S	S	I	I	2
Chrysomus ruficapillus	*Escherichia coli*	S	S	I	S	S	S	S	S	I	S	I	0
	Salmonella ser. Panama	R	R	R	R	S	S	R	R	R	R	I	8
Turdus albicollis	*Escherichia coli*	S	S	R	S	S	S	S	S	S	S	I	1
	Klebsiella	R	S	I	I	S	S	S	S	S	S	I	1
Estrilda astrild	*Escherichia coli*	I	I	R	S	S	S	S	R	I	I	I	2
Tangara sayaca	*Escherichia coli*	S	I	R	S	S	S	S	R	I	I	I	2
Volatinia jacarina	*Escherichia coli*	S	S	I	S	S	S	S	S	S	S	I	0
Sporophila frontalis	*Escherichia coli*	S	S	I	S	S	S	S	R	S	S	I	1
Sporophila falcirostris	*Escherichia coli*	S	S	I	S	S	S	S	S	S	S	S	0
Tangara ornata	*Escherichia coli*	S	R	R	S	R	I	I	R	S	R	I	5
Turdus rufiventris	*Escherichia coli*	S	I	S	S	S	S	I	S	S	S	I	0
Sporophila caerulescens	*Escherichia coli*	S	I	R	S	S	I	R	S	S	I	S	2
Tangara ornata	*Escherichia coli*	S	R	S	S	S	I	S	S	S	S	R	2
	Klebsiella	R	S	S	I	S	I	S	S	S	S	R	2
Saltator similis	*Escherichia coli*	I	R	R	R	S	S	S	S	S	I	R	4
Tangara sayaca	*Escherichia coli*	S	R	S	R	S	S	I	S	S	I	I	2
	Klebsiella	R	S	R	S	S	S	S	S	S	S	I	2
Volatinia jacarina	*Escherichia coli*	S	R	R	S	R	I	I	R	S	R	I	5
Paroaria dominicana	*Escherichia coli*	R	R	R	S	R	R	S	R	S	I	R	7
Tachyphonus coronatus	*Escherichia coli*	S	R	R	S	S	S	I	R	S	I	I	3
Turdus rufiventris	*Escherichia coli*	I	R	R	I	S	S	S	S	S	I	I	2
Tangara ornata	*Escherichia coli*	S	S	R	S	S	S	S	S	S	S	S	1
Volatinia jacarina	*Escherichia coli*	R	S	R	R	R	I	S	S	S	S	I	4
Sicalis flaveola	*Escherichia coli*	R	R	R	R	R	I	I	S	S	S	S	5
Sicalis flaveola	*Escherichia coli*	I	S	R	R	R	S	I	S	S	S	S	3
Sicalis flaveola	*Escherichia coli*	I	S	S	R	R	S	S	S	S	S	S	2
Paroaria dominicana	*Escherichia coli*	I	S	S	R	S	I	S	S	S	S	S	1
Chrysomus ruficapillus	*Escherichia coli*	R	S	R	I	R	I	I	R	S	R	I	5
Turdus rufiventris	*Escherichia coli*	R	S	R	S	S	S	I	S	S	I	I	2
Ramphocelus bresilius	*Escherichia coli*	R	S	R	I	S	I	I	S	S	S	I	2
Tangara sayaca	*Escherichia coli*	I	I	R	S	S	I	I	S	S	S	I	1

– Table 3 (*Continued*)

Host species	Bacterial isolate	Antibiotic resistance											Total
		AMP	CRO	CEF	TCY	SXT	CHL	GEN	NAL	CIP	ENR	NIT	
Sporophila frontalis	*Escherichia coli*	I	I	R	S	S	S	I	S	S	S	I	1
Sicalis flaveola	*Escherichia coli*	R	I	R	R	S	S	I	S	S	S	I	3
Saltator similis	*Escherichia coli*	R	I	R	I	S	I	R	R	S	R	I	5
Paroaria dominicana	*Escherichia coli*	I	S	R	I	R	R	R	R	S	S	I	5
Tangara ornata	*Escherichia coli*	I	S	S	S	S	S	S	S	S	S	R	1
Chrysomus ruficapillus	*Escherichia coli*	R	R	R	S	S	S	I	S	I	R	R	5
	Salmonella ser. Panama	S	R	R	S	S	S	R	S	S	I	I	3
Silcalis flaveola	*Klebsiella*	R	R	R	S	S	S	S	S	S	S	R	4
Sporophila falcirostris	*Salmonella* ser. Typhimurium	I	R	R	R	S	S	S	R	S	R	I	5
	Total	28	21	43	17	17	6	8	17	4	12	19	

AMP, ampicillin; CRO, ceftriaxone; CEF, ceftiofur; TCY, tetracycline; SXT, sulfamethoxazole/trimethoprim; CHL, chloramphenicol; GEN, gentamicin; NAL, nalidixic acid; CIP, ciprofloxacin; ENR, enrofloxacin; NIT, nitrofurantoin; S, no resistance; I, intermediate; R, resistant.

to ceftiofur and ceftriaxone, which are third generation cephalosporins. Ceftiofur is used in veterinary medicine, while ceftriaxone is prescribed in the treatment of severe human *Salmonella* infections.

The presence of antibiotic resistance in wildlife may be a proof of the impact of human activities on natural ecosystems.[40] Wild birds may acquire and disseminate enteric bacteria, including resistant strains, by the fecal–oral route through species that act as carriers, such as insects, rodents and other birds, apart from the contact with human waste and contaminated food. In these cases, they act as reservoirs, carriers or sentinels of resistant bacterial pathogens.[16,36,41,42]

Multiresistant phenotypes in wild bird feces represent an important evidence of the transmission of pathogens and of antimicrobial resistance mechanisms, both domestically and across international borders, that are fostered by the trade of wild animals and a close contact with humans. Additional studies in natural environments, which should include the microbiological monitoring of professionals that directly manage wild animals, are essential to better understand the source of resistant strains isolated from wildlife.

Conflicts of interest

The authors declare no conflicts of interest.

REFERENCES

1. Ferreira CM, Glock L. Preliminar diagnosis of the trafficked avifauna in the state of Rio Grande do Sul, Brazil. *Biociencias*. 2004;12:21–30.
2. Lima R. O tráfico de animais silvestres. In: RENCTAS, ed. *Vida silvestre: o estreito limiar entre preservação e destruição - Diagnóstico do tráfico de animais silvestres na Mata Atlântica: Corredores Central e Serra do Mar*. Brasília: Dupligráfica; 2007:44–49.
3. Ribeiro LB, Silva MG. O comércio ilegal põe em risco a diversidade das aves no Brasil. *Ciênc Cult*. 2007;59:4–5.
4. Alves RRN, Lima JRF, Araujo HFP. The live bird trade in Brazil and its conservation implications: an overview. *Bird Conserv Int*. 2012:1–13.
5. Mittermeier RA, Fonseca GAB, Rylands AB, Brandon K. A brief history of biodiversity conservation in Brazil. *Magadiversity*. 2005;1:14–21.
6. Matias CAR, Oliveira VM, Rodrigues DP, Siciliano S. Summary of the bird species seized in the illegal trade in Rio de Janeiro, Brazil. *TRAFFIC Bull*. 2012;24:83–86.
7. Redford KH. The empty forest. *BioScience*. 1992;42:412–422.
8. Chomel BB, Belotto A, Meslin F. Wildlife, exotic pets, and emerging zoonoses. *Emerg Infect Dis*. 2007;13:6–11.
9. Alves RRN, Nogueira EEG, Araújo HFP, Brooks SE. Bird-keeping in the Caatinga, NE Brazil. *Hum Ecol*. 2010;38:147–156.
10. Fernandes-Ferreira H, Mendonça SV, Albano C, Ferreira FS, Alves RRN. Hunting, use and conservation of birds in Northeast Brazil. *Biodivers Conserv*. 2012;21:221–244.
11. Karesh WB, Cook RA, Bennett EL, Newcomb J. Wildlife trade and global disease emergence. *Emerg Infect Dis*. 2005;11:1000–1002.
12. Hubálek Z. An annotated checklist of pathogenic microorganisms associated with migratory birds. *J Wildl Dis*. 2004;40:639–659.
13. Tsiodras S, Kelesidis T, Kelesidis I, Bauchinger U, Falagas ME. Human infections associated with wild birds. *J Infect*. 2008;56:83–98.
14. Benskin CM, Wilson K, Jones K, Hartley IR. Bacterial pathogens in wild birds: a review of the frequency and effects of infection. *Biol Rev Camb Philos Soc*. 2009;84:349–373.
15. Kruse H, Kirkemo A, Handeland K. Wildlife as source of zoonotic infections. *Emerg Infect Dis*. 2004;10:2067–2072.
16. Steele CM, Brown RN, Botzler RG. Prevalences of zoonotic bacteria among seabirds in rehabilitation centers along the pacific coast of California and Washington, USA. *J Wildl Dis*. 2005;41:235–244.
17. Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH, eds. *Manual of Clinical Microbiology*. 6th ed. Washington, DC: ASM Press; 1995.
18. Murray PR, Baron EJ, Jorgensen JH, Landry ML, Pfaller MA, eds. *Manual of Clinical Microbiology*. 9th ed. Washington, DC: ASM Press; 2007.
19. Grimont PAD, Weill FX. *Antigenic Formulae of the Salmonella Serovars*. 9th ed. WHO Collaborating Centre for Reference and Research on Salmonella; 2007.

20. CLSI (Clinical and Laboratory Standards Institute). *Performance Standards for Antimicrobial Susceptibility: Twenty-second Information Supplement – Document M100-S23*. Wayne, PA: Clinical and Laboratory Standards Institute; 2013.
21. Almeida PM, Arais LR, Andrade JR, Prado EH, Irino K, Cerqueira AM. Characterization of atypical enteropathogenic *Escherichia coli* (aEPEC) isolated from dogs. *Vet Microbiol*. 2012;158:420–424.
22. Gopee NV, Adesiyun AA, Caesar K. A longitudinal study of *Escherichia coli* strains isolated from captive mammals, birds and reptiles in Trinidad. *J Zoo Wildl Med*. 2000;31:353–360.
23. Tizard I. Salmonellosis in wild birds. *Semin Avian Exot Pet Med*. 2004;13:50–66.
24. Abulreesh HH, Goulder R, Scott GW. Wild birds and human pathogens in the context of ringing and migration. *Ringing Migr*. 2007;23:193–200.
25. Bessa MC, Costa M, Cardoso M. Prevalence of *Salmonella* sp. carrier pigs in slaghterhouses of Rio Grande do Sul, Brazil. *Pesq Vet Bras*. 2004;24:80–84.
26. Loureiro ECB, Marques NDB, Ramos FLP, Reis EMF, Rodrigues DP, Hofer E. Salmonella serovars of human origin identified in Pará State, Brazil from 1991 to 2008. *Rev Pan-Amaz Saúde*. 2010;1:93–100.
27. Kich JD, Coldebella A, Morés N, et al. Prevalence, distribution, and molecular characterization of Salmonella recovered from swine finishing herds and a slaughter facility in Santa Catarina, Brazil. *Int J Food Microbiol*. 2011;151:307–313.
28. Hofer E, Filho SJS, Reis EMF. Prevalence of *Salmonella* serovars isolated from birds in Brazil. *Pesq Vet Bras*. 1997;17:55–62.
29. Rabsch W, Andrews HL, Kingsley RA, et al. Salmonella enterica serotype Typhimurium and its host-adapted variants. *Infect Immun*. 2002;70:2249–2255.
30. Kapperud G, Stenwig H, Lassen J. Epidemiology of *Salmonella typhimurium* O:4-12 infection in Norway. *Am J Epidemiol*. 1998;147:774–782.
31. Refsum T, Vikoren T, Handeland K, Kapperud G, Holstad G. Epidemiologic and pathologic aspects of *Salmonella typhimurium* infection in passerine birds in Norway. *J Wildl Dis*. 2003;39:64–72.
32. Thornley CN, Simmons GC, Callaghan ML, et al. First incursion of *Salmonella enterica* serotype Typhimurium DT160 into New Zealand. *Emerg Infect Dis*. 2003;9:493–495.
33. Tsubokura M, Matsumoto A, Otsuki K, Animas SB, Sanekata T. Drug resistance and conjugative R plasmids in *Escherichia coli* strains isolated from migratory waterfowl. *J Wildl Dis*. 1995;31:352–357.
34. Nascimento AMA, Cursino L, Gonçalves-Dornelas H, Reis A, Chartone-Souza E, Marini MA. Antibiotic-resistant gram-negative bacteria in birds from the Brazilian Atlantic Forest. *The Condor*. 2003;105:358–361.
35. Hasan B, Sandegren L, Melhus A, et al. Antimicrobial drug-resistant *Escherichia coli* in wild birds and free-range poultry, Bangladesh. *Emerg Infect Dis*. 2012;18:2055–2058.
36. Carroll D, Wang J, Fanning S, McMahon BJ. Antimicrobial resistance in wildlife: implications for public health. *Zoon Public Health*. 2015;62:534–542.
37. Greig J, Rajic A, Young I, Mascarenhas M, Waddell L, LeJeune J. A scoping review of the role of wildlife in the transmission of bacterial pathogens and antimicrobial resistance to the food chain. *Zoon Public Health*. 2015;62:269–284.
38. Silva-Hidalgo G, López-Valenzuela M, Juárez-Barranco F, Montiel-Vázquez E, Valenzuela-Sánchez B. Salmonella serovars and antimicrobial resistance in strains isolated from wild animals in captivity in Sinaloa, Mexico. *Jpn J Vet Res*. 2014;62:129–134.
39. Berendonk TU, Manaia CM, Merlin M, et al. Tackling antibiotic resistance: the environmental framework. *Nat Rev Microbiol*. 2015;13:310–317.
40. Blanco G, Lemus JA, Grande J, et al. Geographical variation in cloacal microflora and bacterial antibiotic resistance in a threatened avian scavenger in relation to diet and livestock farming practices. *Environ Microbiol*. 2007;9:1738–1749.
41. Hilbert F, Smulders FJM, Chopra-Dewasthaly R, Paulsen P. Salmonella in the wildlife–human interface. *Food Res Int*. 2012;45:603–608.
42. Wellington EMH, Boxall ABA, Cross P, et al. The role of the natural environment in the emergence of antibiotic resistance in Gram-negative bacteria. *Lancet Infect Dis*. 2013;13:155–165.