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Detection of bacteria and fungi and assessment of the molecular aspects and resistance of *Escherichia coli* isolated from confiscated passerines intended for reintroduction programs

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ABSTRACT

Many native bird species are currently considered rare in Brazil because they have been indiscriminately collected by animal traffickers and commercialized, leading to dwindling numbers in their natural habitats. Confiscated animals are at times destined for reintroduction programs that must ensure these animals do not pose a risk to native populations. Healthy or sick wild passerines may carry a great diversity of microorganisms. Therefore, knowledge of the sanitary status of confiscated animals destined for reintroduction is critical to assess whether these animals act as microorganism carriers and to investigate the epidemiology of transmissible diseases, a crucial aspect for animal and human health preservation. This study examined the occurrence of aerobic and facultative anaerobic bacteria and fungi in cloacal swabs collected from wild confiscated passerines intended for reintroduction programs. *In vitro* susceptibility tests of the most frequent isolates as well as studies of the molecular aspects of *Escherichia coli* isolates were also performed. There was microorganism growth in 62.5% of 253 samples. The microorganisms that were most frequently isolated were *Staphylococcus* spp. (15.0%), *Micrococcus* spp. (11.5%), *E. coli* (10.7%) and *Klebsiella* spp. (10.7%). Fifteen bacteria genera and seven fungi genera were isolated. Multidrug-resistance to antimicrobials was observed in *Staphylococcus* spp., *Micrococcus* spp., *E. coli* and *Klebsiella* spp. isolates. The high occurrence of Enterobacteria observed is possibly related to the sanitary conditions in which confiscated animals are usually kept. One *E. coli* sample (out of 27 isolates) was positive for the S-fimbrial adhesion encoding gene (*sfa*). Considering the low occurrence of genes that encode virulence factors, confiscated passerines may represent a low risk for the potential transmission of EPEC, APEC, UPEC and NMEC isolates to other animals or humans. The potential risk of intra- or inter-specific transmission of multidrug-resistant isolates and the introduction of these microorganisms into the environment must be considered, although there are still therapeutic alternatives for treatment of these animals among the antimicrobials which were tested. The stress and poor hygiene conditions imposed on animals during trafficking may have caused their contamination by multidrug-resistant agents transmitted by humans or by the precarious environment to which they were subjected. Risks related to the dissemination of *Salmonella* spp., *Cryptococcus* spp. and *Candida* spp. are low when reintroduction programs are considered.

1. Introduction

Brazil is a country that is rich in wildlife, and the extremely diverse bird fauna that is widely distributed over the country is particularly conspicuous. There are approximately 1901 bird species in Brazil, of which 1064 are passerines [1]. These birds have

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been a constant target of illegal commerce, which can reduce their population numbers. Wildlife trafficking is the third largest illicit activity in the world [2], and Brazil is an important source of animals for trafficking. Birds are the main target of the illegal animal trade, and wild passerines are the most trafficked birds [2].

Confiscated birds are at times destined for reintroduction programs, which consist of an "attempt to establish a population in an area that was once part of the range of the species but from which it has become extirpated" [3], and special attention should be given to ensure that the birds do not pose a risk to native populations. Thus, reintroduction programs must ensure that reintroduced animals are free of microorganisms that can be transmitted to animals native to the release area, which could pose a risk to the health of these animals and thus compromise the conservation goal.

Birds can transmit diseases to humans, to other birds, to domestic animals and to animals native to the area of destination of the reintroduction programs. Therefore, knowledge of the epidemiology of transmissible diseases is critical to preserve human and animal health [4].

Healthy or sick wild passerines may carry a great diversity of microorganisms, including bacteria, viruses and fungi [5]. An assessment of the microorganisms present in wild passerines and of the risk that the microorganisms may pose for the birds themselves and for humans and other animals is of great importance because it can help reduce disease emergence and contribute to biodiversity conservation. Therefore, studies on the sanitary status of confiscated animals that will be reintroduced can be important tools for evaluating whether these animals act as carriers of pathogenic agents to other animals and humans.

Given the scarcity of studies in this area, the present study assessed the occurrence, frequency and characteristics of aerobic and facultative anaerobic bacteria and fungi in cloacal swabs collected from confiscated wild passerines destined for reintroduction programs. The study aimed to better understand the epidemiology of diseases, assist in the implementation of more effective management practices, and improve the surveillance focused on these animals.

2. Materials and methods

2.1. Sample collection

A total of 253 confiscated passerines belonging to 34 distinct species that were sent to the Department of Parks and Green Areas of São Paulo, Brazil – Division of Veterinary Medicine (DEPAVE) were evaluated (Table 1). DEPAVE is an important center for receiving animals confiscated from the trafficking in Brazil, these animals originating from all over the country. São Paulo is also the greatest trafficked animals trade center of the country. Samples were collected through cloacal swabs. The swabs were placed in Stuart's transport medium and sent to the laboratory under refrigeration.

2.2. Isolation and identification of microorganisms present in the cloacal swabs of passerines

To examine the aerobic and facultative anaerobic bacteria, the swabs were first inoculated in brain and heart infusion (BHI) broth and incubated at 37 °C for 24 h. The samples were also inoculated on 5% sheep blood agar and MacConkey agar plates and were incubated aerobically at 37 °C, with readings at 24–96 h. The samples grown in BHI broth were subsequently inoculated on 5% sheep blood agar and MacConkey agar plates and were similarly incubated aerobically at 37 °C with readings at 24–96 h. For the study of *Salmonella* spp., the swabs were inoculated in tetrathionate broth and incubated at 37 °C for 24 h. The samples were also inoculated onto xylose-lysine-tergitol4 (XLT4) agar and were incubated aerobically at 37 °C with readings at 24–96 h. After incubation, the samples transferred to tetrathionate broth were inoculated onto xylose-lysine-tergitol4 (XLT4) agar and were incubated aerobically at 37 °C, with readings at 24–96 h. The bacteria isolated were identified according to their macro- and microscope characteristics and by biochemical tests as described by Murray et al. [6].

For the detection of filamentous fungi and yeasts, the samples were inoculated in Sabouraud dextrose broth and Sabouraud dextrose agar with chloramphenicol (100 mg/l) and incubated at 25 °C for three and seven days, respectively. The agar was evaluated daily. After three days of incubation, the samples grown in Sabouraud dextrose broth were plated onto Sabouraud dextrose agar with chloramphenicol (100 mg/l) and incubated at 25 °C for 7 days. The filamentous fungi and yeasts isolated were identified according to their macro- and microscope characteristics and by physiological tests, as described by Barnett and Hunter [7] and Barnett et al. [8].

The RapID® identification system (Thermo Fisher Cientific®) was used when the results needed to be confirmed.

2.3. In vitro susceptibility testing of the most frequently isolated bacteria to different antimicrobials

The most frequently isolated bacteria were subjected to *in vitro* susceptibility tests to the following antimicrobials: amikacin (30 μg), amoxicillin/clavulanic acid (10 μg), ampicillin (10 μg),

Table 1
List of the passerine species evaluated, number of birds of each species and percentage of birds in relation to the 253 total birds.

Species name	Number of birds	% In relation to the total number of birds
Saltator similis	58	22.9
Sicalis flaveola	36	14.2
Paroaria dominicana	28	11.1
Sporophila caerulescens	23	9.1
Cyanoloxia brissonii	17	6.7
Gnorimopsar chopi	12	4.7
Sporophila frontalis	12	4.7
Turdus rufiventris	10	4
Thraupis sayaca	7	2.8
Zonotrichia capensis	6	2.4
Sporophila falcirostris	5	2
Icterus jamacaii	4	1.6
Sporophila sp.	3	1.2
Sporagra magellanica	3	1.2
Sporophila albogularis	2	0.8
Sporophila nigricollis	2	0.8
Sporophila angolensis	2	0.8
Sporagra magellanica × *Sicalis flaveola*[a]	2	0.8
Turdus leucomelas	2	0.8
Turdus amaurochalinus	2	0.8
Lanio pileatus	2	0.8
Tachyphonus coronatus	2	0.8
Volatinia jacarina	2	0.8
Tangara ornata	1	0.4
Turdus flavipes	1	0.4
Dacnis cayana	1	0.4
Turdus albicollis	1	0.4
Chrysomus ruficapillus	1	0.4
Icterus pyrrhopterus	1	0.4
Paroaria coronata	1	0.4
Sporophila bouvreuil	1	0.4
Euphonia chalybea	1	0.4
Saltatricula atricollis	1	0.4
Sporophila lineola	1	0.4
Total	253	100

[a] Hibrid.