BRAZILIAN JOURNAL OF MICROBIOLOGY

http://www.bjmicrobiol.com.br/

Veterinary Microbiology

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

Carlos Alexandre Rey Matias[a,c,*], *Ingrid Annes Pereira*[b], *Eliane Moura Falavina dos Reis*[b], *Dália dos Prazeres Rodrigues*[b], *Salvatore Siciliano*[d]

[a] *Universidade Federal Rural do Rio de Janeiro, Instituto de Veterinária, Departamento de Epidemiologia e Saúde Pública, Seropédica, RJ, Brazil*

[b] *Fundação Oswaldo Cruz, Instituto Oswaldo Cruz, Laboratório Nacional de Referência de Enteroinfecções Bacterianas, Rio de Janeiro, RJ, Brazil*

[c] *Fundação Oswaldo Cruz, Escola Nacional de Saúde Pública Sérgio Arouca, Programa de Pós-graduação em Saúde Pública e Meio Ambiente, Rio de Janeiro, RJ, Brazil*

[d] *Fundação Oswaldo Cruz, Instituto Oswaldo Cruz, Rio de Janeiro, RJ, Brazil*

ARTICLE INFO

Article history:
Received 30 April 2015
Accepted 6 March 2016
Available online 26 July 2016

Associate Editor: Miliane Moreira Soares de Souza

Keywords:
Antibiotic resistance
Enterobacteriaceae
Public health
Salmonella
Wild birds

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.

* *Corresponding author.*
E-mail: camatias75@gmail.com (C.A. Matias).

http://dx.doi.org/10.1016/j.bjm.2016.07.012

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.