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CIÊNCIAS BIOLÓGICAS

BACTERIA PRESENT IN SCARLET IBIS (*Eudocimus ruber*) CHICKS, BABITONGA BAY, SANTA CATARINA STATE, BRAZIL

BACTÉRIAS PRESENTES EM FILHOTES DE GUARÁ (Eudocimus ruber), BAÍA BABITONGA, ESTADO DE SANTA CATARINA, BRASIL

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Abstract
Wild birds are important for public health because of their potential to transmit pathogenic microorganisms to humans. The waterbird scarlet ibis (*Eudocimus ruber*) forages and breeds near urban areas and if they settle near polluted waters, the viability of adults and their young can be negatively affected. Hence, the aim of this study was to evaluate the cloacal aerobic bacteria profile of nestling scarlet ibis in a mixed colony in Jarivatuba Island, in Joinville, Santa Catarina, Brazil. Cloacal swab samples were collected from clinically normal scarlet ibis nestlings during the breeding season of 2015/2016 (n=16) and 2016/2017 (n=34), and plated onto blood, MacConkey, and *Salmonella-Shigella* agar plates. *Escherichia coli*, *Proteus vulgaris, Proteus* spp., *Klebsiella* sp., *Enterococcus* spp. and *Staphylococcus* spp. were isolated and may be representative of the normal microbiota of *E. ruber*, although the normal profile is unknown for the species. However, the location of this colony in an area without adequate sewage treatment, which receives domestic effluents, may indicate a modified bacterial profile. Further studies are needed, to better understand the host's natural microbiome, as well as on the bacterial isolates, in order to characterize any association with the contaminated water. These results lay the foundation for successful species conservation projects in the area by providing insights that will help improve the viability of nestlings in each reproductive season.
Keywords: Pelecaniformes, scarlet ibis, nestlings, bacteria.

Resumo
Aves silvestres são importantes para a saúde pública devido ao seu potencial de transmissão de microrganismos patogênicos aos seres humanos. As aves aquáticas, como o guará (*Eudocimus ruber*), forrageiam e se reproduzem próximo de áreas antropizadas e estas, quando contaminadas, podem transmitir bactérias patogênicas às aves. O objetivo deste trabalho foi identificar o perfil de bactérias aeróbicas cloacais em filhotes de guará na colônia mista da Ilha Jarivatuba, em Joinville, Santa Catarina. Foram coletados *swabs* cloacais de filhotes de guará na estação reprodutiva de 2015/2016 (n=16) e 2016/2017 (n=34), todos de aves de aspecto clínico normal, e plaqueados em ágar sangue, MacConkey e *Salmonella-Shigella* em aerobiose. Foram isolados os seguintes microrganismos:

Escherichia coli, *Proteus vulgaris, Proteus* spp., *Klebsiella* sp. *Enterococcus* spp. e *Staphylococcus* spp. A ocorrência de *E. coli*, *Enterococcus* spp. e *P. vulgaris* podem ser representantes da microbiota natural da espécie, uma informação desconhecida. Entretanto, a localização da colônia de aves aquáticas, na foz do rio Cachoeira, com o aporte de efluentes domésticos e industriais da cidade de Joinville sem tratamento adequado, pode indicar modificação dos perfis bacterianos. Torna-se evidente a necessidade de avançar com a sua tipificação fenotípica e genotípica dos isolados, para análises comparativas com estirpes presentes nos efluentes sanitários da região. Os resultados poderão contribuir para a conservação da espécie e outras aves aquáticas da área, permitindo a elaboração de projetos de conservação, gestão do ambiente costeiro e marinho, além de subsidiar medidas preventivas efetivas no combate as perdas de filhotes e potenciais epidemias zoonóticas.
Palavras-chave: Pelecaniformes, guará, filhotes, bactérias.

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Introduction

Zoonoses are diseases that affect both animals and humans[1]. However, information on their etiology and pathogenesis in wild animals is scarce[2,3], especially when it comes to less charismatic vertebrates and invertebrates, such as marine animals[1]. Wild birds are important for public health because they can be infected with microorganisms that can be transmitted to humans[4,2]. They are known to be reservoirs of several agents, including arbovirus, Nile virus, influenza A virus, pathogenic bacteria, and antibiotic-resistant bacteria[1,4]. Among the bacteria that are known to be transmitted from wild birds to humans with the possibility of causing disease, are *Escherichia coli*, *Borrelia burgdorferi*, *Anaplasma phagocytophilum*, *Salmonella typhimurium*, *Campylobacter* spp., and *Mycobacterium* spp.[5].

The primary source of infection in birds is the oral-fecal route, through the ingestion of contaminated food and water and direct contact with infected animals[6]. Birds are vulnerable to pathogen infections at all stages of their life cycle, both before and after hatching[2]. Although the eggshell acts as a physical barrier against some microorganisms, many bacteria can penetrate the pores of the shell and membrane, thus infecting the egg contents[7]. After hatching, chicks may contract infections through possibly contaminated food offered by their parents [8]. Chicks of colonial birds occur at high densities and are, therefore, vulnerable to the exchange of disease pathogens until they leave the nest[2,8].

Research involving the microbiota of wild birds are scarce or limited to the trade and trafficking of Passeriformes and Psittaciformes, or to a small number of species, mainly those closely associated with humans such as pigeons and seagulls[2,3]. Many authors believe that the role of birds as transmitters of bacterial pathogens may have been underestimated[2]. Birds can cover long distances, which, consequently, allows the dispersion of the microorganisms they carry.

Waterbirds are at the top of the food chain. They forage in the aquatic environment and are therefore susceptible to contamination by bacteria from untreated domestic wastewater. Consequently, they can be reservoirs of infection[9]. Thus, waterbirds' health directly reflects on human health, since these birds are considered as bioindicators of environmental changes[10].

Scarlet ibis (*Eudocimus ruber*) is a waterbird of the order Pelecaniformes and family

Threskiornithidae that inhabits mangroves, swamps, and the swampy savannah (Llanos) of the Southern hemisphere[11]. The species is described as a tactile predator, inserting its long beak into the soil in search of prey, where it feeds mainly on crustaceans[12,13]. Scarlet ibis foraging sites can be located in anthropic areas and when these areas are contaminated by sewage, solid waste, and domestic animal feces, the species becomes susceptible to bacterial pathogen infections.

Castelo-Branco et al. (2017)[14] examined whether captive scarlet ibis (*Eudocimus ruber*) in Mangal das Garças Park (Belém-PA) are reservoirs and carriers of *Aeromonas* spp. and *Plesiomonas* spp. These bacteria are described as constituents of the microbiota of ectothermic animals and waterbirds, and are potentially pathogenic in humans, causing waterborne diseases[15]. *Aeromonas veronii* bv. *sobria*, *Aeromonas hydrophila,* and *Plesiomonas shigelloides* bacterial species were isolated in the analyses. The research demonstrated that scarlet ibis could act as reservoirs of fish and human pathogens due to their diet, based on crustaceans and fish. Monitoring of the environment occupied by these birds is extremely important due to the possibility of transmission of diseases by water and food[14].

Information on the health of scarlet ibis is scarce[14], especially when it comes to wild individuals. Therefore, this study aimed to identify bacteria associated with scarlet ibis in a reproductive colony in Babitonga Bay, north coast of Santa Catarina.

Material and Methods

The scarlet ibis colony studied is located on Jarivatuba Island (26º29′66.45′′S, 48º79′58.14′′W), Babitonga Bay, with an area of about 136,645 m². It is a recently formed island, with several islets covered in mangrove, which are influenced by tidal variations[16]. The island is located in the city of Joinville, the largest city in Santa Catarina State, where only 31% of sewage is treated[17]. The largest nesting site of Babitonga Bay is found on this island. Besides the scarlet ibis, other species of waterbirds breeding there include the black-crowned night heron (*Nycticorax nycticorax*), yellow-crowned night heron (*Nyctanassa violacea*), cattle egret (*Bubulcus ibis*), great egret (*Ardea alba*), snowy egret (*Egretta thula*), little blue heron *(Egretta caerulea*) and white-faced ibis (*Plegladis chihi*).

The study area was visited weekly between September 2015 and March 2016 (2015/2016 season), and between September 2016 and March 2017 (2016/2017 season) to monitor the reproductive cycle of scarlet ibis. For the bacteriological analyses, apparently healthy stage 2 chicks (chicks more than two weeks old whose sex could not be visually distinguished) were captured. The criterion used for choosing nests was their height in the trees; nests closer to the ground were selected because they were more accessible to the researchers. A 4 m aluminum ladder was used to reach nests and chicks were captured manually or using a hand net and were placed inside cloth bags to reduce stress. Chicks were weighed using a Pesola® scale, with a precision of 10 g. Total length was measured using a metal ruler. For sample collection, a swab with Cary-Blair agar was introduced into the cloaca and rotated for 30 sec. The chicks were then banded with metal rings provided by the National Center for Bird Conservation (CEMAVE) and received a combination of colored rings, which allowed identification of each individual chick from nests with more than one chick. The nests were marked with plastic seals with numbers. At the end of the procedure, the chick was returned to its nest. Swabs

were sent to the laboratory for bacterial culture within 24 h.

The samples from the 2015/2016 breeding season were plated in Petri dishes containing blood agar (NewProv)®, MacConkey agar and *Salmonella-Shigella* (Prodimol Biotecnologia)® using a semiquantitative technique with an inoculation loop. After this procedure, the plates were incubated in an oven at 35°C for 24 h. All plaques that showed bacterial growth were analyzed for colony morphology, bacterial morphology by Gram staining, and phenotypic evidence for each genus.

The samples from the following year (2016/2017 breeding season), were plated in Petri dishes containing MacConkey agar and *Salmonella-Shigella* (Laborclin)® using a qualitative technique with a disposable inoculation loop. The plates were then incubated in a bacteriological oven at 35°C for 24 h. The plaques that showed bacterial growth were analyzed for colony morphology and staining. The staining properties (Gram) and bacterial morphology of all colonies were evaluated. Gram-negative bacteria that grew in Mac Conkey agar were plated using an EMP/MILI (Laborclin)® kit for identification of enterobacteria. Gram-positive cocci were tested for catalase, and subsequent phenotypic tests were performed for each genus.

The licenses issued to conduct the research were SISBIO no. 49541-1, CEMAVE no. 4014/1 and Univille Research Animals Ethics Committee (Opinion no. 006/2015).

The frequency of occurrence (FO) was calculated from the number of samples in which each bacterium was isolated / total number of samples × 100.

Results

In the 2015/2016 breeding season, 16 cloacal swab samples were collected and analyzed to verify the presence of microorganisms. The mean weight of the chicks was 320.8 g, and the mean total body length was 26.7 cm. The following microorganisms were isolated: *Escherichia coli*, *Proteus vulgaris,* and *Klebsiella* sp. Four samples had two bacterial isolates, eight samples had only one isolate, and in four samples no bacteria grew (Table 1). The bacterium with the highest frequency of occurrence in the samples was *E. coli* (Figure 1).

In the 2016/2017 breeding season, 34 scarlet ibis chicks were captured and ringed. The mean weight was 364.4 g, and the mean total body length was 27.5 cm. A total of 34 cloacal swabs were analyzed, and at least four bacteria were isolated: *E. coli*, *Enterococcus* spp., *Staphylococcus* spp., and *Proteus* spp.. Seven samples had three bacterial isolates, and 27 samples had two bacterial isolates (Table 2). *Escherichia coli* was isolated from all samples (Figure 2).

Table 1. Bacteria isolated in cloacal swab samples of scarlet ibis (*Eudocimus ruber*) (Birds: Pelecaniformes) in Jarivatuba Island, Rio Cachoeira, Babitonga Bay (Joinville, Santa Catarina, Brazil) in the 2015/2016 breeding season. Identification of the bird (ring number), weight (grams) and size (total body length in centimeters).

Identification of the bird	Weight (g)	Size (cm)	Bacterial isolate
T43318	325	28	*Escherichia coli*
T43319	335	24	*Escherichia coli, Proteus vulgaris*
T43320	214	22	*Escherichia coli*
T43321	144	17	*Escherichia coli, Proteus vulgaris*
T43323	198	19	*Escherichia coli*
T43324	340	29	*Escherichia coli, Proteus vulgaris*
T43325	295	25	*Escherichia coli, Proteus vulgaris*
T43329	212	22	Absent
T43330	300	22	Absent
T43331	425	29	*Escherichia coli*
T43332	395	27	Absent
T43333	375	34.5	*Klebsiella* sp.
T43334	385	33	*Klebsiella* sp.
T43335	330	30	*Escherichia coli*
T43336	480	36	*Escherichia coli*
T43337	380	30	Absent

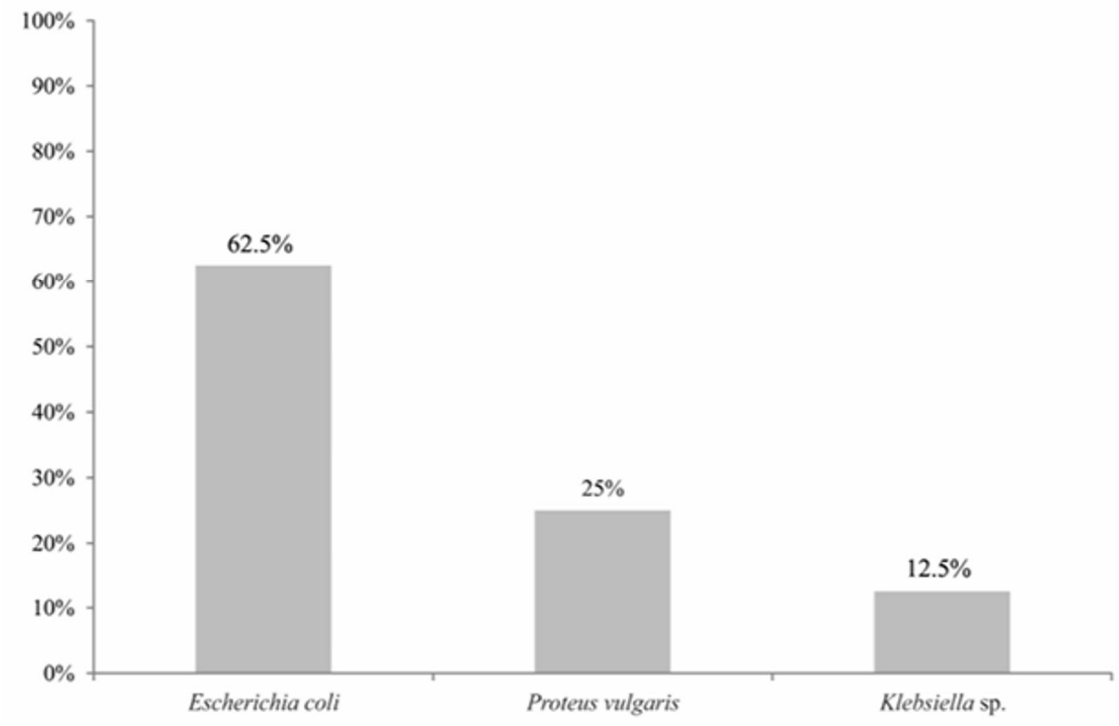

Figure 1. Bacterial isolates and their frequency of occurrence from cloacal swab samples of scarlet bis (*Eudocimus ruber*) in the 2015/2016 breeding season in Jarivatuba Island, Babitonga Bay (Joinville, Santa Catarina, Brazil).

Table 2. Bacteria isolated from cloacal swab samples of scarlet ibis (*Eudocimus ruber*) (Birds: Pelecaniformes) on Jarivatuba Island, Rio Cachoeira, Babitonga Bay (Joinville, Santa Catarina, Brazil), in the 2016/2017 breeding season. Identification of the bird (ring number), weight (grams) and size (total body length in centimeters).

Identification of birds	Weight (g)	Size (cm)	Bacterial isolate
T43338	380	29	*Escherichia coli, Staphylococcus* spp., *Proteus* spp.
T43339	410	30	*Escherichia coli, Staphylococcus* spp.
T43340	335	24	*Escherichia coli, Enterococcus* sp., *Proteus* spp.
T43341	340	23	*Escherichia coli, Staphylococcus* spp.
T43342	330	27	*Escherichia coli, Staphylococcus* spp., *Proteus* spp.
T43343	385	26	*Escherichia coli, Staphylococcus* spp., *Proteus* spp.
T43344	370	28	*Escherichia coli, Staphylococcus* spp., *Proteus* spp.
T43345	260	24	*Escherichia coli, Staphylococcus* spp.
T43346	495	34.5	*Escherichia coli, Staphylococcus* spp., *Proteus* sp.
T43347	580	32	*Escherichia coli, Enterococcus* spp.
T43348	315	29	*Escherichia coli, Enterococcus* spp.
T43349	400	27	*Escherichia coli, Staphylococcus* spp.
T43350	335	25	*Escherichia coli, Enterococcus* spp.
T43351	345	27.5	*Escherichia coli, Enterococcus* spp.
T43352	470	34	*Escherichia coli, Enterococcus* spp.
T43353	320	23	*Escherichia coli, Enterococcus* spp.
T43354	355	30	*Escherichia coli, Enterococcus* spp.
T43355	320	25	*Escherichia coli, Enterococcus* sp.
T43356	298	24.5	*Escherichia coli, Enterococcus* sp.
T43357	405	29.5	*Escherichia coli, Enterococcus* sp.
T43358	370	25.5	*Escherichia coli, Enterococcus* sp.
T43359	422	32	*Escherichia coli, Enterococcus* sp.
T43360	380	27	*Escherichia coli, Enterococcus* sp.
T43361	430	30	*Escherichia coli, Proteus* sp.
T43362	315	26	*Escherichia coli, Enterococcus* sp.
T43363	380	30.5	*Escherichia coli, Staphylococcus* sp.
T43364	335	27.5	*Escherichia coli, Proteus* sp.
T43365	330	25	*Escherichia coli, Staphylococcus* sp., *Proteus* sp.
T43366	390	28	*Escherichia coli, Proteus* sp.
T43367	410	29.5	*Escherichia coli, Proteus* sp.
T43368	295	25	*Escherichia coli, Enterococcus* sp.
T43369	330	28.5	*Escherichia coli, Proteus* sp.
T43370	245	23	*Escherichia coli, Proteus* sp.
T43371	310	27	*Escherichia coli, Proteus* sp.

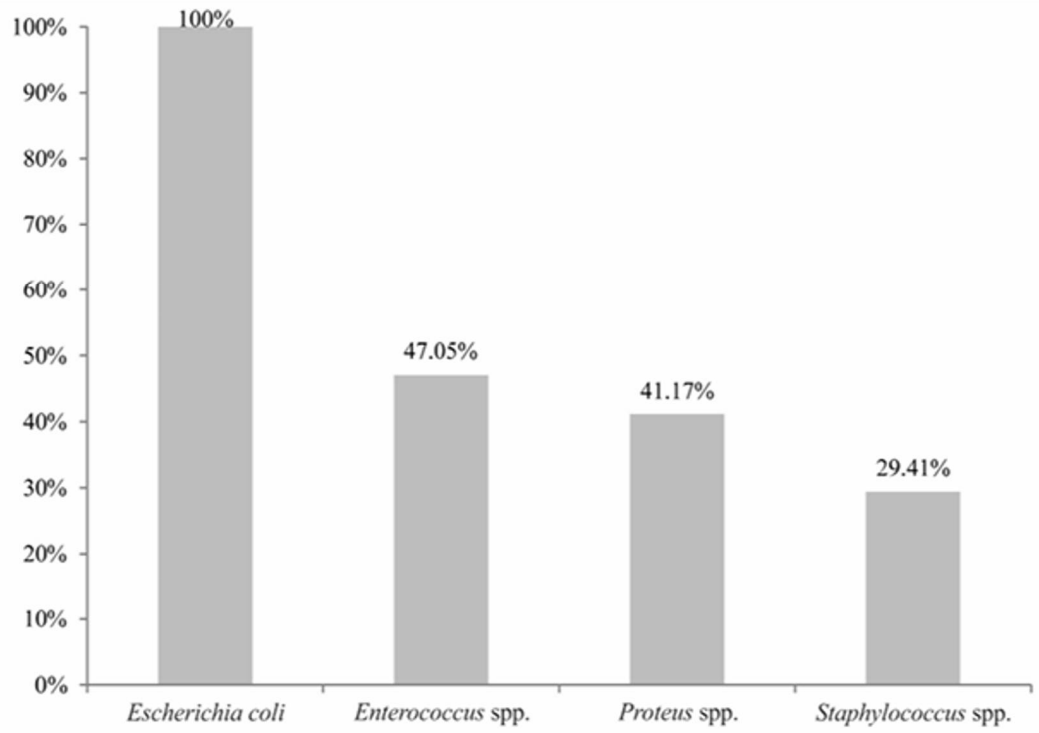

Figure 2. Bacterial isolates and their frequency of occurrence from cloacal swab samples of scarlet ibis (*Eudocimus ruber*) in the 2016/2017 breeding season in Jarivatuba Island, Babitonga Bay (Joinville, Santa Catarina, Brazil).

Discussion

The difference in the number of bacteria isolated between the two years of study may be due to natural changes in the feeding and habitat of scarlet ibis. We observed that in the 2015/2016 breeding season, some cloacal samples of scarlet ibis did not show any bacterial growth, while in the 2016/2017 season, at least two isolates were identified in each of the samples. Castelo-Branco et al.[14] observed that in scarlet ibis, habitat and feeding could be responsible for the bacteriological composition, which reinforces the importance of environmental monitoring.

Five genera of bacteria were observed in total. Of these, three were Gram-negative and two were Gram-positive. In similar studies on seabirds, more bacterial species were isolated from cloacal samples: thirteen species of bacteria were identified in gull chicks (*Larus dominicanus*) in the Tamboretes islands[18] and nineteen species of bacteria were found in brown boobies (*Sula leucogaster*) in the same location[19]. Identification of bacteria in wild birds is a first step to distinguish commensal and pathogenic bacteria, which can be influenced by food, habitat, and other infected birds[18].

Considering that the environment studied is affected by sewage, it is possible that all the *E. coli*, *Enterococcus*, *Proteus*, and *Staphylococcus* isolates found in the cloaca of *E. ruber* chicks were of human origin. The genetic characterization of the isolated strains could allow the determination of the host species.

Escherichia coli isolates were detected in all samples in the 2016/2017 breeding season and 62.5% of the 2015/2016 samples. This bacterial species has been considered as non-pathogenic for a long time[20] since it is normally found in the intestinal tract and mucosa of warm-blooded animals, which

also occur in the environment, and it is eliminated in feces and can contaminate water and food[21,22]. However, some serogroups have evolved to acquire different sets of virulence genes, classified as diarrheagenic and extra-intestinal serotypes, becoming pathogenic for humans and animals[20,23].

Escherichia coli infections of the APEC group (Avian Pathogenic *E. coli*) are related to an extra-intestinal disease in birds called colibacillosis. This disease starts with respiratory tract infections, or air sacculitis, evolving into a generalized infection that can take the form of polyserositis, pericarditis, perihepatitis, or peritonitis[21,10]. Infections caused by *E. coli* affecting captive crested ibis (*Nipponia nippon*) caused sepsis, sudden death, anorexia, diarrhea, and claudication in six chicks[24]. In humans, it causes diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome[22]. In domestic animals, it can cross the intestinal barrier and cause sepsis, urinary infections, pyometra, and mastitis[20,22].

The high frequency of *E. coli* in this study may be related to the place where the reproductive colony is established, since it is located at the mouth of the Cachoeira River, in the Cachoeira River Basin, which receives most of the municipal and industrial effluents of Joinville[17]. According to Castro-Silva et al.[19], the greater frequency of occurrence of *E. coli* in brown-boobies (19.51%), compared to that of other bacteria, may be associated with the proximity of the Tamboretes islands to Babitonga Bay, which is influenced by the largest industrial center of Santa Catarina (Joinville). Joinville, in turn, is largely responsible for the inadequate disposal of domestic and industrial effluents in the region, since this species bacteria is related to human waste[19].

In Egypt, the frequency of isolated *E. coli* in samples from crested ibis was 43.6%, and of *Salmonella* spp. was 14.5%. The authors attributed necrotic alterations and degeneration of hepatocytes observed in the histological analyses of birds to these bacteria[25]. Other isolated pathogenic bacteria were *Shigella* spp. (34.5%), *Enterobacter* spp. (21.8%), *Citrobacter* spp. (18.1%), *Klebsiella pneumonia* (16.3%), *Staphylococcus aureus* (10.9%), and *Proteus mirabilis* (7.2%)[25]. Suphoronski et al.[26] evaluated the occurrence of *E. coli* and *Salmonella* spp. in wild and captive birds in Paraná state. A total of 69.38% cloacal swab samples were positive for *E. coli,* and 22.32% were positive for *Salmonella* spp.. The research showed that these vertebrates can host and disseminate these pathogens, allowing transmission to humans and other animals, since samples from birds that have close contact with humans, such as those of the Columbiformes order (pigeons), were the ones which had the highest occurrence of *E. coli* (82.33%)[26].

Salmonella spp. was not detected in the analysis of the cloacal samples of scarlet ibis. However, this pathogen must be investigated, as it causes salmonellosis, an important global zoonosis that can cause harm to human and animal health[22] and lead to a loss of wildlife[10]. According to Gilchrist[27], the isolation of *Salmonella* spp. could be inhibited by the common contamination of cultures by *Citrobacter* and *Proteus* enterobacteria. Furthermore, the release of *Salmonella* usually occurs when the bird is stressed or immunosuppressed, since this bacterium remains protected within macrophages in viscera, such as the liver and spleen[28].

The *Proteus* genus includes Gram-negative, facultative anaerobic bacteria, which are considered opportunistic pathogens in humans, and are isolated in urine, wounds, and other clinical sources[29]. They can also be hosted by domestic and wild animals, acting as a parasite or commensal[29]. Their presence may indicate water and soil fecal pollution[29]. This genus was identified in the scarlet ibis chicks in both study years. In the Tamboretes islands, *Proteus mirabilis* was isolated in 16.51% of the gull cloacal samples and *Proteus vulgaris* was in 3.67%[18] of the samples. Isolated *Proteus* found in the cloaca of *E. ruber* chicks may be of human origin.

Enterococcus spp. were some of the most recurrent bacteria in the 2016/2017 season samples. They are Gram-positive bacteria, are isolated in feces, water, soil, plants, and food products, and are present in the gastrointestinal tracts of humans and animals[30]. Despite their low virulence, they are emerging as important pathogens, which are resistant to medically important antibiotics and chemicals released into the environment. Moreover, urban pigeons can act as reservoirs and contribute to their propagation[31].

Staphylococcus are Gram-positive bacteria, and although they act primarily in endogenous infections, they may also act opportunistically in respiratory diseases in birds[32]. They were isolated in 29.41% of the 2016/2017 season samples. *Staphylococcus* bacteria had the highest occurrence in the cloacal samples of brown boobies collected in the Moleques do Sul islands[19].

Klebsiella sp. had the lowest frequency of occurrence in the present study (12.5%). Bacteria of this genus are associated with fecal pollution[19]. They are opportunistic enterobacteria that affect stressed and immunosuppressed birds, and may cause respiratory and renal problems and, in chronic infections, may attack the lungs[32]. Cloacal swabs from 253 Passeriformes confiscated from trafficking and illegal trade and destined for reintroduction programs,[3] revealed *Staphylococcus* spp. (15%), *Micrococcus* spp. (11.5%), *E. coli* (10.7%), and *Klebsiella* spp. (10.7%). Stress and poor sanitary conditions may compromise the immunity of these birds[3].

Evaluating the pathogenicity level of bacteria isolated from scarlet ibis chicks, using more accurate methodologies for the classification of these microorganisms, is of paramount importance. There is a clear need to conduct further studies analyzing other bird species, as the results will contribute to the management of the coastal and marine environment, as well as to the promotion of effective preventive measures to combat possible pathogen epidemics.

Conclusion

The bacteria isolated from the cloacal samples of scarlet ibis chicks were previously described in other species of birds. Some strains of these microorganisms may be potentially pathogenic to wild birds, threatening their conservation, as well as posing risks to domestic species and humans. Therefore, it is important that further health studies be conducted for the conservation of scarlet ibis and other waterbirds that nest in the colony, including studies of parasitic and toxic infectious agents in the affected environment.

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