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Detection of Pathogenic Bacteria Isolated from *Larus dominicanus* on the Coast of Brazil

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ABSTRACT

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Bacterial resistance is a significant public health issue as it exacerbates adverse impacts on human, animal, and environmental health. *Larus dominicanus* are considered potential carriers or reservoirs. The objective of this study was to identify the possible presence of bacteria pathogenic to humans in the gull *L. dominicanus* and to evaluate their resistance to antibiotics. For this, 39 cloacal samples were obtained from *L. dominicanus* with swabs and subjected to biochemical tests for the detection of gram-positive and gram-negative bacteria; the isolated bacteria were subjected to antibiogram analysis using the cutoff points reported in the Clinical and Laboratory Standards Institute *Performance Standards for Antimicrobial Susceptibility Testing*. Twenty-two bacteria were isolated from the cloacal samples, with three families and 12 distinct genera with zoonotic potential observed more frequently in the gulls of the Moleques do Sul Archipelago. The antibiogram revealed that most of the bacteria were resistant to ampicillin.

ADDITIONAL INDEX WORDS: *Seabird, microbiota, antibiotics, bacterial resistance, public health, zoonosis.*

INTRODUCTION

Antibiotics, since the discovery of penicillin and its use during World War II, have become a milestone in the advancement of medicine because fatal diseases were and are easily treated by the availability of various drugs on the market today (Weckx, 2012). The use of these drugs has been responsible for reducing the rates of morbidity and mortality associated with bacterial infections worldwide (Orús *et al.*, 2015). However, the indiscriminate and often abusive use of these drugs has generated an adaptive response of bacteria through mechanisms capable of making them resistant to antibiotics, reducing or even nullifying their effects (De Oliveira and Da Silva, 2008). This bacterial resistance is a risk to human and animal health, representing a public health problem (Del *et al.*, 2017). Bacterial resistance has reduced the number of effective drugs for the treatment of various diseases, causing clinical complications, increasing the length of hospital stays, and increasing the number of deaths (Santos, 2004). Thus, the World Economic Forum Global Risks classified bacterial resistance as one of the major threats to human health (Blair *et al.*, 2015; Vásquez-García *et al.*, 2019).

Enterobacteriaceae is a family of biochemically similar bacteria that are gram negative, rod shaped, aerobic (facultative anaerobes), and largely motile due to the presence of flagella (Lister and Barrow, 2008). This family includes genera that are relevant to human and animal health, such as *Salmonella* sp., *Escherichia* sp., *Shigella* sp., *Citrobacter* sp., *Klebsiella* sp., *Proteus* sp., and *Yersinia* sp. (Doi, De Oliveira, and Barbieri, 2015; Lister and Barrow, 2008; Mignani *et al.*, 2013). The World Health Organization warns that bacteria of the *Enterobacteriaceae* family that are resistant to antibiotics should be considered a priority because they are responsible for increased human morbidity and mortality and are zoonotic, being easily transmitted between humans, animals, and the environment (Hadjadj *et al.*, 2017).

The *Enterococcaceae* family is composed of gram-positive bacteria, and the widely studied genus *Enterococcus* includes 52 species and two subspecies (Parte, 2018). This genus is a facultative aerobe, has a short-chain coccus morphology, and is present in the intestinal microbiota of mammals (including humans, birds, and fish) (Lory, 2014) in addition to the soil and water (Porto *et al.*, 2016). The *Micrococcaceae* family is represented by the genus *Staphylococcus*, which comprises 49 different species, and nasal microbiota (Jay, 2000).

Birds are organisms widely affected by bacterial infections and can transmit zoonoses to humans (Guimarães, 2007). As an example, the bacterium *Escherichia coli*, present in the intestinal microbiota of homeothermic animals, can cause intestinal and extraintestinal diseases in humans, mammals, and birds, characterizing a public health problem (Bélanger *et al.*, 2011). Salmonellosis is an important bacterial disease of birds with great zoonotic potential and high mortality (Kanashiro *et al.*, 2002). *Enterococcus faecalis* and *Enterococcus faecium* cause systemic diseases in birds and humans, especially in hospitalized patients, resulting in difficult treatment and a

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significant increase in deaths (O'Driscoll and Crank, 2015). Bacteria of the genus *Staphylococcus* are also noteworthy, causing medical concern in hospitals because they cause serious infections in humans, such as pneumonia, meningitis, and endocarditis (Dos Santos *et al.*, 2007).

Studies that demonstrate the occurrence and distribution of pathogenic bacteria in humans are important for detecting possible hosts responsible for transmission such as birds (Beleza *et al.*, 2019), which are one of the main pathways responsible for the spread of antibiotic-resistant bacteria (Bartley *et al.*, 2019; Huijbers *et al.*, 2015). Wild birds, especially aquatic and migratory birds, are considered carriers or potential reservoirs of several pathogens and may thus play an important role in the epidemiologic chain of diseases because of their ability to fly over several regions in a short period of time (Silva *et al.*, 2011). Seagulls inhabit coastal regions around the world and are protected by various environmental laws. Although their diet is based on fish and crustaceans from the sea and beaches along the coast, during recent decades, numerous authors have demonstrated the ability of gulls to use food sources of human origin, such as urban landfills and fishing grounds, to the detriment of the ease of obtaining them from traditional food reserves (Barbieri, 2008; Ebert, Branco, and Barbieri, 2014, 2020; Frere, Gandini, and Martinez, 2000; Miotto *et al.*, 2017).

In Brazil, one of the most abundant and well-adapted gulls is *Larus dominicanus*. This is one of the most common species of seabirds along the coast and continental lands of the Atlantic, occurring in Brazil, the Antarctic Peninsula, the Pacific coast of South America and southwest Africa, Australia, and New Zealand (Branco, Fracasso, and Barbieri, 2009). This species is considered opportunistic and generalist in its feeding habits, being able to use a diversity of prey and explore different food sources (Ebert and Branco, 2009). In addition, it occupies several nesting environments, such as islands, beaches, dunes, and coastal lagoons, interacting with species of seabirds (Ebert and Branco, 2009) and with humans.

The closer relationship between gulls and humans may represent a risk for the development of diseases, including bacterial diseases, in which the gull represents a source of bacterial infection harmful to human health. Another important issue of this close relationship, which deserves attention, is the favoring of the selection of antibiotic-resistant bacteria due to contact with remains of drugs present in the garbage disposed of every day in urban centers because these animals are present in cities (Del *et al.*, 2017).

Notably, pathogenic bacteria are responsible for several serious infections in humans, such as bloodstream infections, pneumonia, and complicated urinary tract and intra-abdominal infections (Ting, Lee, and Liu, 2018). This situation may be intensified if the infection is caused by environmental strains that are resistant to antibiotics (Koneman *et al.*, 2008), resulting in significant clinical and socioeconomic impacts (Sheu *et al.*, 2019).

Studying the bacterial microbiota of clinically healthy wild birds is essential for understanding the epidemiology of bacterial diseases that may affect their populations and related species, providing a critical tool for disease prevention (Dobbin *et al.*, 2005). Identifying potential sources of pathogenic bacterial infections in humans, particularly those associated with seabirds like *L. dominicanus*, is thus crucial. This study aims to identify bacteria in water gulls (*L. dominicanu*s) that may cause zoonotic diseases and act as reservoirs of antibiotic-resistant strains. Thus it was hypothesized that seagulls are carriers of bacteria that are possibly pathogenic to humans, with the potential to be resistant to antibiotics, due to their characteristics such as (1) having the ability to travel long distances in a short time; (2) being in places with human contact; and (3) being close to landfills and human waste.

METHODS

During a period of approximately 16 months (12 June 2012 to 20 October 2013) cloacal samples from the gull *L. dominicanus* were collected in the archipelagos of Moleques do Sul, Tamboretes, and Ilha dos Lobos, located off the coast of Santa Catarina, Brazil (Figure 1) with cotton swabs. The technique is not invasive and does not cause any sort of stress or mortality for these birds. As a research team we got permission from the IBAMA–Chico Mendes Institute for Preservation in Brazil linked by the Ministry of Environment.

The Moleques do Sul Archipelago is considered the main nesting site of seabirds on the coast of Santa Catarina and is part of the Serra do Tabuleiro Park and therefore protected by environmental laws that guarantee reproduction without human contact (Branco, Fracasso, and Barbieri, 2009). The island of Lobos is owned by the Brazilian Navy, with its name attributed to the presence of sea lions. On the island, vegetation is minimal, and its characteristics and proximity to the city of Laguna make it an important location for seabirds including *L. dominicanus* (Figure 2).

Chicks were captured in nests because they could not yet fly or run away. It was not possible to catch the adult gulls. Thirty-nine cloacal samples were obtained with swabs: 17 from the Moleques do Sul Archipelago, 13 from the Tamboretes Archipelago, and 9 from Ilha dos Lobos (Table 1). The samples were immediately stored in a tube containing Cary Blair transport culture medium. The number of samples and the collection period differed among the archipelagos because of adverse weather conditions.

The samples were kept in the transport tube at room temperature and sent to a laboratory within 2 days after collection. At the laboratory, the samples were conditioned in peptone water and kept at 35°C. After 48 hours, the samples were transferred to tetrathionate broth, targeting species of the genus *Salmonella*, for 24 hours at 35°C. The samples were then seeded on plates containing *Salmonella–Shigella*, xylose lysine deoxycholate, and bismuth sulfite culture media. After 24 hours, Gram staining was performed to observe morphology, and the colonies were subcultured on trypticase soy agar. The samples were also seeded in mannitol salt culture medium for the detection of gram-positive bacteria.

The following biochemical media were used to identify the species of gram-negative bacteria: Simmons citrate agar, triple sugar iron agar, broth urea agar, SIM agar, and Rugai lysine agar. To identify the species of gram-positive bacteria, the following means were used: DNase agar, coagulase (tube), catalase (slide), and the pyrrolidonyl arylamidase test (Table 2).

All samples, after seeding in enrichment broths and conducting biochemical tests, remained for 48 hours at 35°C and

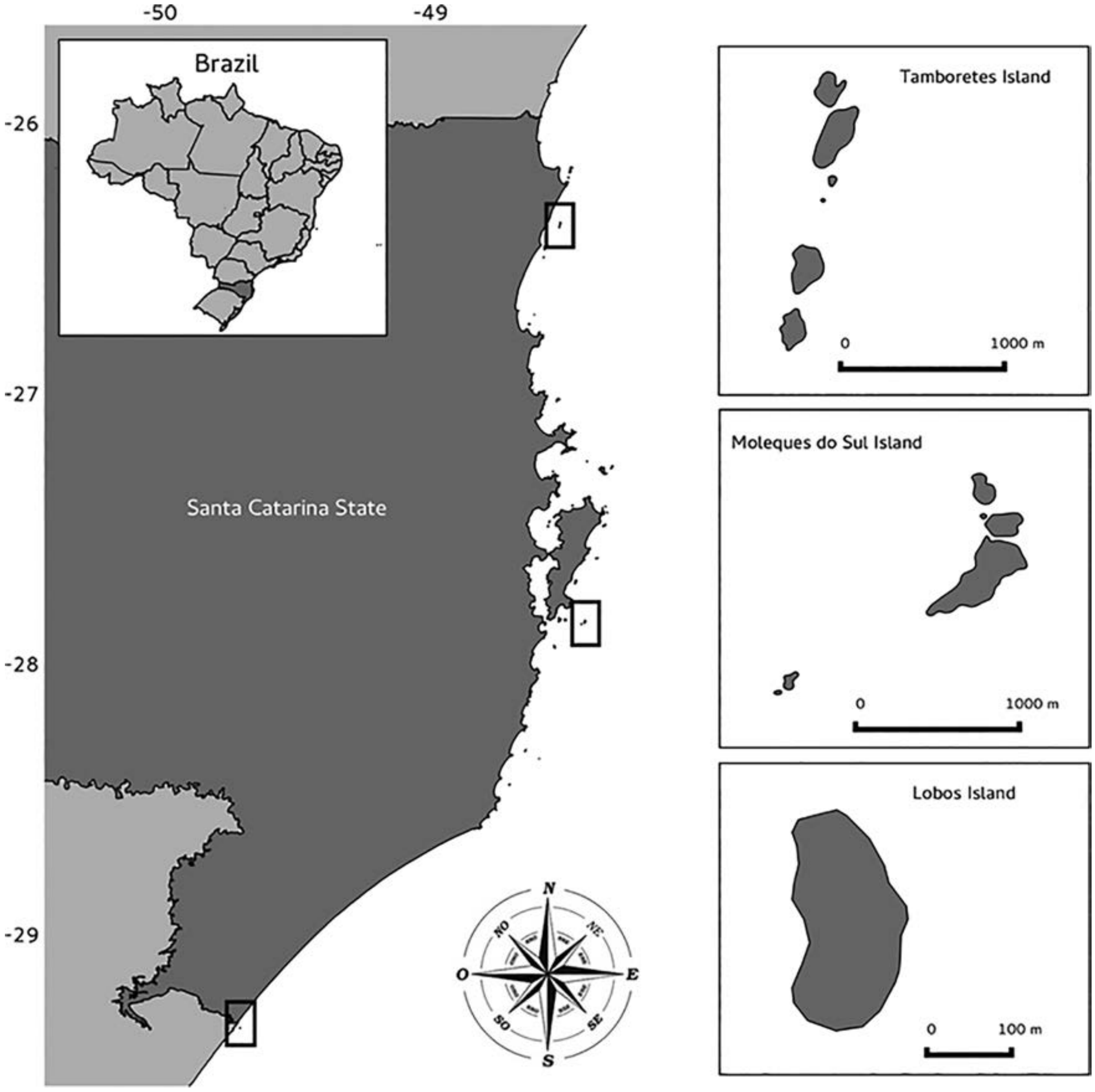

Figure 1. Islands along the coast of Santa Catarina.

then remained for 24 hours in selective culture media. For the identification of the bacteria, the macroscopic characteristics of the culture media used, according to the manufacturer, as well as the biochemical results, were analyzed.

After the identification of the bacterial species, antibiograms were performed following the Clinical and Laboratory Standards Institute (2022; Table 3) and using the respective cutoff points for antibiotics to provide an updated view of the resistance profile of these bacteria. These samples were incubated at 35°C for 48 hours and the halo measurement in millimeters was used to determine the resistance/sensitivity of the bacterial species.

RESULTS

Twenty-two bacteria belonging to three families and 12 distinct genera, including gram- negative and gram-positive bacteria, were isolated from the cloacal samples of *L. dominicanus* (Table 4). In the three archipelagos, the main gram-positive bacteria were *Staphylococcus aureus* and *Staphylococcus epidermidis*, with a prevalence of approximately 20.5% among the samples. Most enteric bacteria with zoonotic potential were in the Moleques do Sul Archipelago, an area protected because it is part of the Serra do Tabuleiro National Park, where there are no significant levels of contamination by human waste and food nearby (Table 5). Regarding the antibiograms, the bacteria identified in the Moleques do Sul and Tamboretes archipelagos showed a high level of sensitivity to most antibiotics. Most resistance to the tested antibiotics was found with ampicillin (AMP), with some cases of resistance to aztreonam (ATM) and piperacillin, especially for the genus *Citrobacter* (Table 6).

Figure 2. Adult and chick of *Larus dominicanus*.

DISCUSSION

The gulls of *L. dominicanus*, present in the archipelagos of Santa Catarina, Brazil, proved to be a reservoir of gram-positive and gram-negative bacteria possibly pathogenic to humans. Gargiulo et al. (2018) emphasize that wild birds serve as reservoirs and potential vectors of infection, promoting the development of antibiotic-resistant strains. As gull populations are commonly observed in urban areas near coastal regions, this species may contribute to the transmission of resistant strains of pathogenic microorganisms across species, including humans and economically significant animals.

The contamination of gulls *L. dominicanus* in the Moleques do Sul archipelago may indicate that these birds are increasingly entering urban areas for food and thus contacting contaminants of human origin (dumpsters, sewage, and treatment plant waste, among others). The observation of high levels of potentially pathogenic bacteria leads to hypotheses involving environmental contamination. In the studied region, this issue is significant because these islands are located within environmentally protected areas and parks. The bacteria found may be part of the natural intestinal microbiota of the birds, posing no direct harm to the birds or their environment. However, the detection of *S. aureus* and *S. epidermidis* in cloacal samples from the gull *L. dominicanus* in this study raises concerns. If pathogenic to this bird species, these bacteria could lead to diseases such as acute septicemia and chronic osteomyelitis (Skeeles, 1997). Moreover, their presence poses potential risks to human health.

Table 1. *Number of samples and corresponding percentage from each collection site: Moleques do Sul Archipelago, Tamboretes Archipelago, and Ilha dos Lobos.*

Archipelagos	Number of Samples	Percentage of Samples (%)
Moleques do Sul	17	43.59
Tamboretes	13	33.33
Ilha dos Lobos	9	23.08
Total	39	100

Table 2. *List of biochemical tests used to detect gram-negative and gram-positive bacteria in cloacal samples from Larus dominicanus.*

Gram Negative	Gram Positive
Motility	Catalase
Indole	Coagulase
Triple Sugar Iron Agar (Glucose/Lactose)	DNase
Gas (CO_2)	Pyrrolidonyl Arylamidase
H_2S	
Lysine Citrate	
Urea	

In addition, bacteria of the *Enterobacteriaceae* family are known to cause many infectious diseases, especially diarrheal and dysenteric syndromes, accompanied by typhoid fever, which is caused by *Salmonella* and *Shigella* species (Koneman *et al.*, 2008). The genus *Salmonella* has reptiles, birds, and mammals (including humans) as reservoirs and is known worldwide as a food contaminant that causes diseases such as gastroenteritis and typhoid fever, leading to high morbidity and mortality, both in the wild animal population and in the human population (Silva *et al.*, 2010).

Although *Escherichia coli* and bacteria of the genus *Staphylococcus* can cause food poisoning, bacteria of the genus *Salmonella* are the most frequently isolated from outbreaks that cause human pathologies (Chomel, Belotto, and Meslin, 2007). *Salmonella* Enteritidis Paratyphi A and *Salmonella* Choleraesuis are among the *Salmonella* types of greatest epidemiological importance. Both types of bacteria are important causes of infectious diseases and may be able to mutate and resist antibiotics, for example, through gulls, which serve as a reservoir of multidrug-resistant strains. The intracellular viability of the microorganism, the production of cytotoxins, the presence of endotoxins, and the resistance to conventional antimicrobials are among the main virulence factors associated with infections by the different *Salmonella* serotypes in animals (Ribeiro *et al.*, 2010).

Bacteria such as *Es. coli, Proteus* spp., and members of the *Klebsiella–Enterobacter* group are commonly isolated from traumatic wounds, contaminated soil and plant material, and abdominal incisions after gastrointestinal surgery (Koneman *et al.*, 2008). In addition, the presence of bacteria such as *Yersinia pseudotuberculosis* and *Klebsiella pneumoniae* reinforces the theories that these birds serve as a reservoir and

Table 3. *List of antibiotics used to measure the resistance/sensitivity of gram-negative bacteria (Enterobacterales), Staphylococcus spp., and Enterococcus spp.*

Gram Negative	*Staphylococcus* spp.	*Enterococcus* spp.
CIP	IPM	PG
IPM	AMP	CIP
TE	VA	TE
GEN	TE	MPA
ATM	CIP	VA
AMP	OX	IPM

AMP = ampicillin, ATM = aztreonam, CIP = ciprofloxacin, GEN = gentamicin, IPM = imipenem, OX = oxacillin, PG = penicillin G, TE = tetracycline, VA = vancomycin

Table 4. *Family, genus, and species identified in the six samplings (39 samples) at the three archipelagos.*

Family	Genus/Species	Gram	NA	DA (%)	OT	NT (%)
Enterobacteriaceae	*Citrobacter amalonaticus*	–	1	0.03	1	0.01
	Citrobacter freundii	–	2	0.05	6	0.05
	Citrobacter koseri	–	4	0.10	11	0.09
	Enterobacter aerogenes	–	1	0.03	2	0.02
	Enterobacter agglomerans	–	1	0.03	1	0.01
	Enterobacter cloacae	–	1	0.03	1	0.01
	Escherichia coli	–	4	0.10	2	0.02
	Klebsiella pneumoniae	–	2	0.05	4	0.03
	Leclercia adecarboxylata	–	1	0.03	1	0.01
	Proteus mirabilis	–	2	0.05	18	0.15
	Proteus vulgaris	–	3	0.08	4	0.03
	Providencia rettgeri	–	1	0.03	1	0.01
	Salmonella Choleraesuis	–	1	0.03	1	0.01
	Salmonella Enteritidis (group)	–	1	0.03	5	0.04
	Salmonella Enteritidis Paratyphi A biotype	–	3	0.08	14	0.12
	Salmonella Typhi	–	1	0.03	4	0.03
	Shigella spp.*	–	3	0.08	7	0.06
	Yersinia pseudotuberculosis	–	1	0.03	1	0.01
	Yersinia spp.*	–	1	0.03	1	0.01
Enterococcaceae	*Enterococcus* spp.*	+	2	0.05	4	0.03
Micrococcaceae	*Staphylococcus aureus*	+	6	0.15	28	0.24
	Staphylococcus epidermidis	+	2	0.05	7	0.06

**spp. = unidentified species, only genus*

Columns show the gram-positive (+) or gram-negative (–) stain of the bacteria, total number of bacteria of that type (NA), value in percentage of types (DA), total number of bacterial types obtained on trypticase soy agar (TSA) plates identified by biochemical tests (OT), and value in percentage of the bacteria identified on TSA plates—117 plates total (NT).

source of infection and foster the development of antibiotic-resistant strains. Studies show that *Yersinia* spp., when inoculated intragastrically into humans, can invade various organs and tissues, where they remain for variable periods, proliferating or not (De Medeiros, Shimizu, and Falcão, 1987; Lister and Barrow, 2008).

Table 5. *Bacteria isolated from cloacal samples from Larus dominicanus in the different archipelagos: Moleques do Sul (AM), Ilha dos Lobos (IL), and Tamboretes (AT).*

Bacteria	AM	IL	AT
Citrobacter amalonaticus			x[†]
Citrobacter freundii			x
Citrobacter koseri	x	x	x
Enterobacter aerogenes	x		
Enterobacter agglomerans	x		
Enterobacter cloacae		x	
Enterococcus spp.			x
Escherichia coli	x		x
Klebsiella pneumoniae	x		x
Leclercia adecarboxylata	x		
Proteus mirabilis		x	x
Proteus vulgaris	x		x
Providencia rettgeri		x	
Salmonella Choleraesuis	x		
Salmonella Enteritidis (group)		x	
Salmonella Enteritidis Paratyphi A biotype	x		x
Salmonella Enteritidis	x		
Salmonella Typhi	x		x
Shigella spp.	x		x
Staphylococcus aureus	x	x	x
Staphylococcus epidermidis	x		
Yersinia pseudotuberculosis	x		
Yersinia spp.			x

[†] *x represents the detected presence of the bacterial type in cloacal samples along the islands.*

Leclercia adecarboxylata of the *Enterobacteriaceae* family are gram-negative bacilli that is rarely found in human and animal intestinal microbiota, and in general is not found because of the difficulty of isolation. The mechanisms of virulence and symptoms of this bacterium in humans have not been properly clarified because of few studies on *Leclercia*; however, it can contaminate food, soil, and water and has been reported as an opportunistic pathogen of immunocompromised patients in general and patients with carcinoma (Correa *et al.*, 2012).

Providencia rettgeri are gram-negative motile bacteria in the family *Enterobacteriaceae*. They are opportunistic pathogens in humans and are mainly associated with urinary tract infections, especially in patients with catheters or extensive burns. Bacteria of the *Providencia* genus are also a serious cause of contamination in hospitals, *e.g.*, on probes used in patients (De Sousa *et al.*, 2004).

Many of the samples contained *Es. coli*, gram-negative bacillary enterobacteria common in the permanent microbiota of humans and animals. The level of contamination of birds represents possible anthropic effects (sewage) to which these animals can be exposed, thus becoming reservoirs of strains that can cause diseases and develop drug resistance. The mechanisms of contamination with *Es. coli* make these bacteria an important cause of hospital contamination, and are also important because of their resistance to antibiotics and treatments. Resistant strains are often identified in contaminated hospital environments designed to remain sterile, *e.g.*, intensive care units (Krewer *et al.*, 2012). *Escherichia coli* is one of the species in which multiresistant strains to antimicrobials have emerged rapidly because of its wide environmental distribution and propensity to obtain genetic elements such as plasmids. Antibiotic abuse exacerbates the

Table 6. *Results of the antibiograms.*

Antibiograms of gram-negative bacteria (general; mm)						
Bacteria	AMP	ATM	CIP	GEN	IPM	TE
Citrobacter amalonaticus	SH (R)[†]	15 (R)	28 (S)	27 (S)	28 (S)	SH (R)
Citrobacter freundii	SH (R)	14 (R)	23 (I)	25 (S)	29 (S)	26 (S)
Citrobacter koseri	SH (R)	23 (S)	25 (I)	25 (S)	28 (S)	19 (S)
Enterobacter aerogenes	SH (R)	43 (S)	34 (S)	22 (S)	34 (S)	25 (S)
Enterobacter agglomerans	19 (S)	25 (S)	23 (I)	21 (S)	32 (S)	30 (S)
Enterobacter cloacae	SH (R)	28 (S)	28 (S)	19 (S)	30 (S)	27 (S)
Escherichia coli	12 (R)	30 (S)	32 (S)	18 (S)	24 (S)	22 (S)
Klebsiella	SH (R)	29 (S)	25 (I)	18 (S)	30 (S)	25 (S)
pneumoniae	SH (R)	35 (S)	33 (S)	23 (S)	26 (S)	24 (S)
Leclercia adecarboxylata						
Proteus mirabilis	12 (R)	15 (R)	27 (S)	20 (S)	26 (S)	18 (S)
Proteus vulgaris	18 (S)	24 (S)	39 (S)	27 (S)	31 (S)	20 (S)
Providencia rettgeri	31 (S)	44 (S)	32 (S)	21 (S)	31 (S)	08 (R)
Salmonella Choleraesuis	13 (R)	SH (R)	28 (S)	19 (S)	30 (S)	24 (S)
Salmonella Enteritidis (group)	14 (I)	29 (S)	35 (S)	25 (S)	32 (S)	27 (S)
Salmonella	10 (R)	31 (S)	36 (S)	25 (S)	24 (S)	21 (S)
Enteritidis Paratyphi A	10 (R)	13 (R)	37 (S)	22 (S)	26 (S)	13 (R)
Salmonella Typhi						
Shigella ssp.	20 (S)	22 (S)	21 (R)	27 (S)	35 (S)	27 (S)
Yersinia	SH (R)	14 (R)	24 (I)	20 (S)	30 (S)	17 (S)
Pseudotuberculosis						
Yersinia spp.	SH (R)	21 (S)	28 (S)	27 (S)	30 (S)	25 (S)
Antibiograms of the genus *Enterococcus* spp.						
Bacteria	AMP	CIP	TE	PP	VA	
Enterococcus spp.	6 (R)	22 (S)	12 (R)	19 (S)	22 (S)	
Antibiograms of the genus *Staphylococcus* spp.						
Bacteria	AMP	CIP	IMP	TE	OX	VA
Staphylococcus aureus	SH (R)	23 (S)	13 (*)	20 (S)	16 (R)	18 (*)
Staphylococcus epidermidis	26 (S)	22 (S)	28 (*)	28 (S)	08 (R)	26 (*)

[†] *Resistance / sensitivity indicated by halos following the Clinical and Laboratory Standards Institute (2022).*

**Point value cutoff not defined.*

R = resistant, I = intermediate, S = sensitive, SH = no halos were formed, AMP = ampicillin, ATM = aztreonam, CIP = ciprofloxacin, GEN = gentamicin, IPM = imipenem, PG = penicillin G, TE = tetracycline, VA = vancomycin

development of bacterial resistance, which is increasingly evident for both human and animal pathogens (Krewer *et al.*, 2012).

The indiscriminate use of antibiotics in daily life, combined with the lack of diagnostic tests confirming bacterial infections and the premature discontinuation of antibiotic treatments, creates ideal conditions for the development of the antibiotic resistance seen today. This situation has led to the development of antibiotic-resistant strains against antibiotics such as AMP; 58% of treatments in patients for urinary infections caused by *Es. coli* are not effective if this medication is used (Casas, Ortiz, and Erazo-Bucheli, 2009). According to some studies, this bacterial resistance may occur because of intrinsic characteristics (structural or functional) of the bacteria, mutations, or the acquisition of exogenous genetic material (present in other microorganisms) (Blair *et al.*, 2015). The increase in bacterial resistance, especially among potentially dangerous pathogens, has led to an increase in the need for new drugs and new classes of antibiotics. However, only two new classes of antibiotics have been introduced into medicine since 1963, when nalidixic acid was approved: one in 2000 and another in 2003 (De Brito and Cordeiro, 2012).

The detection of resistant bacteria in gulls on all continents, including critical pathogens such as *Es. coli* and *Sa. enterica* resistant to beta-lactam antibiotics, illustrates the potential of gulls to participate in the alarming global spread of antibiotic resistance (Zeballos-Gross *et al.*, 2021). In this study, most levels of resistance to various antibiotics are explained by intrinsic resistance. For example, *Enterobacter aerogenes*, *Citrobacter koseri*, *Y. pseudotuberculosis*, *K. pneumoniae*, *Citrobacter freundii*, *Citrobacter amalonaticus, Enterobacter cloacae,* and *Yersinia* spp. exhibit intrinsic resistance to AMP (Clinical and Laboratory Standards Institute, 2022), and *P. rettgeri* exhibits intrinsic resistance to tetracycline (TE). This type of resistance is due to bacterial mechanisms involved in the reduction of outer membrane permeability and the natural activity of drug efflux pumps (Reygaert, 2018). However, clinically important genera such as *Salmonella*, *Proteus*, *Leclercia*, and *Escherichia* have levels that are given by the possible acquisition of resistance genes. For example, the species *Sa.* Enteritidis Paratyphi A and *Sa.* Choleraesuis show resistance to AMP, ATM, and TE and AMP and ATM, respectively. This genus is common in the microbiota of gulls in South America, which show resistance to beta-lactams due to the presence of the *CTX-M* gene (Fuentes-Castillo *et al.*, 2019). *Proteus mirabilis* also shows resistance to AMP and ATM. *Citrobacter amalonaticus* shows resistance to ATM and TE. Finally, the bacteria *Sa.* Enteritidis, *Es. coli*, and *L. adecarboxylata* show resistance only to

AMP, which could suggest the presence of beta-lactamase enzymes. Future analyses should be performed to characterize the antibiotic resistance genes for both gram-negative and gram-positive bacteria as well as possible mobile genetic elements present in these pathogens that would better explain the levels of resistance obtained.

Finally, environmental surveillance of pathogens of critical importance in the environment is important through the One-Health concept and through constant monitoring of these pathogens in *L. dominicanus* microbiota samples as possible biomarkers of anthropogenic contamination.

CONCLUSIONS

The importance of knowing the capacity that seabirds, especially *L. dominicanus*, a species common to the coast of Santa Catarina, have as reservoirs of bacteria pathogenic to humans as well as to animals is fundamental for the development of efficient prophylaxis programs to prevent outbreaks of bacterial pathogens. The study of the seabird microbiota provides an important tool for the pathologic and epidemiologic control of bacteria. The presence of species potentially dangerous to human health, such as species of the genus *Salmonella*, combined with the ability of these animals to move along the coast and even into the coastal territory, facilitates the transport of antibiotic-resistant strains and possible human pathogens. This study contributes up-to-date information on the microbiota present in seabirds and the development of bacterial resistance to antibiotics, allowing the creation of efficient prophylactic measures to combat possible pathogenic outbreaks and indicating the need for constant research and updated monitoring of microorganisms in seabirds.

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