

Original Research Article

<http://dx.doi.org/10.20546/ijcmas.2016.505.048>

Pathogenic Bacteria Associated with Kelp Gull *Larus dominicanus* (Charadriiformes, Laridae) on the Coast of Santa Catarina State - Brazil

Luis A. Ebert^{1,2*}, Júlio C. Schlemper¹, Márcia R. Pelisser¹, Bianca de A. Pereira³,
Marcus A.C. da Silva⁴ and Joaquim O. Branco^{4,2}

¹UNIASSELVI, Centro Universitário Leonardo da Vinci, Indaial, Brasil

²DEBE, Departamento de Ecologia e Biologia Evolutiva, Universidade Federal de São Carlos (UFSCar), São Carlos, Brasil

³UDESC, Universidade do Estado de Santa Catarina (UDESC), Lages, Brasil.

⁴CTTMar, Centro de Ciências Biológicas da Terra e do Mar, Universidade do Vale do Itajaí (UNIVALI), Itajaí, Brasil

*Corresponding author

ABSTRACT

Keywords

Larus dominicanus;
pathogenic
bacteria;
seabirds;
Kelp Gull;
microorganisms.

Article Info

Accepted:
18 April 2016
Available Online:
10 May 2016

Seabirds such as *Larus dominicanus* are very vulnerable to contaminants and pathogens and can be used to reflect changes in the health of marine ecosystem. The objective of this study was to identify pathogenic bacteria associated with the Kelp Gulls on the coast of Santa Catarina. Monthly campaigns were made on three coastal islands, where cloacal samples were taken from young gulls and used for microbiota identification. In the laboratory the samples were analyzed, targeting species gram-negative (genus *Salmonella*) and gram-positive (genus *Staphylococcus*). Through specific biochemical tests were identified 23 species. The Kruskal-Wallis test ($H = 74,914$; $n = 33$; $p < 0,05$) indicated significant differences among the islands applied to number of isolated strains per specie. Tamborettes Island presented higher diversity (2.36) of bacteria, followed by Moleques do Sul (2.10) and Lobos Islands (1.35). Moleques do Sul had the highest richness value (2.87), followed by Tamborettes (2.55) and Lobos (1.14). The equitability was higher for Tamborettes (0.92), followed by Moleques do Sul (0.79) and Lobos (0.51). Greater similarity between Tamborettes and Moleques do Sul was observed by Jaccard. However Bray-Curtis indicated greatest association between Lobos and Tamborettes. The results suggest that pathogenic bacteria are associated with young gulls.

Introduction

Seabirds can be used as indicators of quality marine environments (Furness, 1987). In particular, coastal regions are fragile ecosystem and susceptible to anthropogenic exploitation. Thus, in a context-defined biological monitoring, birds stand out as

important biomarkers. Besides their ecological role, seabirds may reflect changes in ecosystems through behavioral changes, eating habits and reproductive aspects. Therefore, they may be used to reflect changes on health marine environments,

being known as sentinels ocean (Boersma *et al.*, 2001).

Within such context, the species *Larus dominicanus* (Lichtenstein, 1823) has become important to the understanding of some of these problems. In recent decades, researchers have observed a significant increase in populations of the kelp gull, as the species is also known. These observations have mainly occurred in coastal areas of North America, Europe, Canada, South Africa, Australia and Argentina (Yorio *et al.*, 1998; Borboroglu and Yorio, 2004; Altwegg *et al.*, 2007). In general, these birds have been able to increase the number of individuals due to their ability to use foods from anthropogenic origin (Yorio *et al.*, 1998), and also to what many researchers have called reproductive plasticity, in which different populations complete their reproductive cycle in distinct times throughout the year (Burger and Gochfeld, 1996).

The kelp gull is found along the coast as well as on continental land throughout the Atlantic, from Brazil to Tierra del Fuego to the Antarctic Peninsula and sub-Antarctic islands. It is also present on the Pacific coast of South America, southwestern Africa, Australia, and New Zealand (Yorio and Quintana, 1996).

This species is also present on the Brazilian coast between the states of Rio Grande do Sul and Espírito Santo (Sick, 1997). Schiefler and Soares (1995) argue that the gull *L. dominicanus* is one of the most common seabirds of Santa Catarina coast, being abundant in beaches, mangroves, islands and coastal lagoons. It is a very opportunistic species regarding feeding and habitat use, thus being able to occupy urbanized environments and also landfills near these (Ebert and Branco, 2009).

The microbiota of birds is affected by many factors. Among such factors, the type of diet, dwelling environment, antibiotic contamination and infection by pathogenic organisms can be highlighted (Palmgren *et al.*, 1997; Lu *et al.*, 2003). Other authors emphasize that diseases in wild birds can be acquired through infectious agents, mainly related to the birds dwelling places and also due to their eating habits and the type of resources available for such purpose (Mills *et al.*, 1999; Waldenstrom *et al.*, 2002; Maul *et al.*, 2005). As the number of individuals of this species near coastal urban areas is increasing, a significant potential for these birds to be regarded as disseminator vectors of pathogenic species to humans is observed. These pathogens can be transmitted via cross contamination, through contact with food and the man (Kelley *et al.*, 1998).

Kelp gulls are member of Chariiformes order, which can be observed in some continents, thus contributing to the spread of diseases and pathogens, carrying them from one region to the other, increasing the contamination areas (Santos *et al.*, 2012). Wild birds, especially migratory waterfowl, are considered carriers or potential reservoirs of several pathogens, and may therefore play an important role in a disease epidemiology chain due to their ability to fly several regions in a short time span (Silva *et al.*, 2011; Benskin *et al.*, 2009).

In Brazil, recent studies have focused on reproduction and distribution (Barbieri 2008; Ebert and Branco, 2009; Branco, 2004; Branco *et al.*, 2009), whereas the main studies elsewhere are related to diet (Schiavini and Yorio, 1995) and the species interaction with the man (Yorio and Quintana, 1996). In New Zealand, a study refers to the presence of nematodes, cestodes and trematodes in the digestive tract of gulls (Twentyman and Alley, 1999).

A very interesting and widely studied aspect concerns the interaction between industrial fishing and seabirds, especially *L. dominicanus*. Some studies address the negative aspects of this interaction, such as the mortality caused by fishing implements. Others approach ecological issues, although these are not yet fully understood (Furness and Monaghan, 1987; Blaber *et al.*, 1995; Furness, 2003; Branco, 2000, 2001, 2003; Skov and Durinck, 2001; Martinez-Abraín *et al.*, 2002; Sullivan *et al.*, 2006; Louzao *et al.*, 2006; Zador *et al.*, 2008). This shows a gap regarding the understanding of other ecological processes involving seabirds which, in an environmental context, would be important information for better management and protection of marine ecosystems.

Currently, studies that seek to understand the relationships between wild birds and the diseases transmission through cross contamination are increasing gradually, reflecting the importance of studies that may show the increase of such problems, particularly for public health. Thus, understanding the bacterial microbiota of clinically healthy wild birds is important for understanding the epidemiology of bacterial diseases that can affect their own populations as well as similar species, being an important means for prophylaxis (Dobbin *et al.*, 2005; Sarker *et al.*, 2012).

This study aimed to identify bacteria species associated with *L. dominicanus* at the Santa Catarina islands, as well as to identify these microorganisms as potential vectors of disease transmission for these seabirds and also humans.

Materials and Methods

Study Area

From May/2011 to November/2013, monthly campaigns were made on three

coastal islands located in the southern Brazil of Santa Catarina state, called Tamboretes, Moleques do Sul and Lobos respectively. The Tamboretes archipelago is composed of the Norte, Porto, Meio and Bird islands and is located on the northern Santa Catarina (26°22'10" S, 48°31'11" W), five kilometers away from the coast approximately.

This archipelago is inserted in the state park called Acaraí, created in 2005. It has 6.667 acres and great diversity of fauna and flora. The Bird Island was used as a representative of archipelago, where all samples were collected. The Moleques do Sul Island (27°50' S, 48°25' W), is located in a central portion of the state of Santa Catarina, 12 kilometers away from the coast of Florianópolis. This island is part of a natural state park called Parque Estadual da Serra do Tabuleiro (Bege and Pauli, 1989; Branco, 2004). The Ecological Reserve of Lobos Island is located between the states of Santa Catarina and Rio Grande do Sul, in front of Laguna city (26°48' S, 48°23' W), and constitutes an important nesting area for the *L. dominicanus* (Figure 1). It should be noted that a management plan for this conservation area has not yet been developed and the site has consistently suffered anthropogenic pressures, such as unsustainable tourism, indiscriminate fishing, and great movement of vessels, besides the risk of contamination by oil and other chemical pollutants (Branco, 2004; Cesconetto *et al.*, 2011).

For this research important to observe that the islands are located at the north, center and south of the Santa Catarina state. Thus, the geographic distribution of these islands was taken into account to obtain representative samples of the entire Santa Catarina coast, and also the biggest colonies of *L. dominicanus* (Figure 1).

Field Activities

Monthly visits to the islands were held between May 2011 and November 2013. Despite the impossibility of capturing adult gulls during their offspring growing period, it was possible to capture immature individuals that were not yet able to fly, from which cloacal samples were obtained and used for microbiota identification. On Moleques do Sul, the field occurred between May 2011 and November 2011 (number of outings = 6), whereas the fieldwork on Tamboretes, located in the north of the state, was conducted between May 2012 and November 2012 (number of outings = 6); on Lobos, in the south, the outings occurred between August and November 2013 (number of outings = 3).

Samples were obtained with the use of cloacal swab containing Cary Blair culture medium for transport. For each sample one young gull was captured. The samples were kept at room temperature, packed in polystyrene boxes and sent to the laboratory within two days on average from the date of collection. In the laboratory, the samples were placed in peptone water and kept at 35°C for 48 hours. Subsequently the samples were transferred to tetrathionate broth, targeting species gram-negative (genus *Salmonella*), and also seeded in mannitol salt agar to obtain gram-positive bacteria (genus *Staphylococcus*). The samples remained in tetrathionate broth for 24 hours at 35°C and were then sown in Petri dishes with *Salmonella Shigella* (SS), Xilose Lisina Desoxicolato (XLD) and Bismuth Sulphite agar (BS agar). After 24 hours of incubation all colonies were purify in TSA agar (trypticase soy agar) for observation of species morphology (Silva *et al.*, 2010).

Simmons' citrate agar (CS agar), Triple sugar iron (TSI agar), Urea broth agar (UB

agar), SIM agar, and Rugai-lysine agar were used to identify Gram-negative bacteria. DNase agar, tube coagulase test, catalase test in blade, and PYR test were used to identify Gram-positive bacteria. All samples remained for 48 hours at 35°C after seeded in enrichment broths and biochemical tests, and 24 hours in selective media. Macroscopic features of the culture media employed were used for identification in accordance with the manufacturers, as well as the biochemical results used (Silva *et al.*, 2010).

Data Analysis

The total number of swabs collected on the islands allowed the inoculation of Petri dishes, from which the frequency among the isolated strains was logged. For data analysis, Kolmogorov-Smirnov was used to observe the normality of data, and then, Kruskal-Wallis test was used to verify the existence of significant differences in the number of isolated strains identified among the islands. The results were presented in form of graphs and charts using Excel 2010 software. The Past 3.0 was used to calculate the diversity (Shannon-Weaver or H'), equitability (Pielou or J') and richness (Margalef or Dmg), as well as the Jaccard and Bray-Curtis similarity index.

Results and Discussion

It was possible to capture 39 young gulls, where cloacal swabs were obtained: 17 samples were obtained in the Moleques do Sul Island, 13 samples in the Tamboretes (Bird Island) and 9 samples on Lobos Island, representing 43.6%, 33.3% and 23.1 % of the total samples respectively. In terms of percentages, the total number of species of bacteria identified by island in the cloacal samples of young gulls accounted were 42.42%, 39.39% and 18.18% respectively (Figure 2).

Regarding the microbiota, a total of 23 species of bacteria belonging to 12 different genera were identified on the islands. On Moleques do Sul, a total of 14 species of pathogenic bacteria were identified, whereas 13 were identified on Tamboretes and 6 on Lobos Island. Of the twenty-three (23) species of bacteria identified, ten (10) were common to two or three islands. The 39 samples collected allowed the inoculation of 117 Petri dishes with specific culture media for the growth of bacterial colonies. Therefore, it was possible to register the number and frequency of isolated strains, according to the species of bacteria (Table 1).

Was not observed a normal distribution for number of isolated strains per species in each island. The Kruskal-Wallis test ($H = 74,914$; $n = 33$; $p < 0,05$) indicated significant differences among the islands applied to number of isolated strains per specie.

From the total number of inoculated dishes, in terms of percentages, as shown in Table 1, of the most representative species that are common to two or three islands, the following stand out: *Staphylococcus aureus* (30.43%, 35.90% and 25.69% - Moleques do Sul Islands, Lobos Islands and Tamboretes Islands, respectively), *Salmonella enteritidis* (20.65%, 24.36% and 17.43% - Moleques do Sul, Lobos and Tamboretes, respectively), *Citrobacter koseri* (11.96%, 14.10% and 10.09% - Moleques do Sul, Lobos and Tamboretes, respectively) and *Shigella* sp. (7.61% and 6.42% - Moleques do Sul and Tamboretes Islands, respectively). For all other species identified, the number of strains was less than 5%, and/or were not common to two or more islands.

Values for diversity (Shannon-Weaver), richness (Margalef), and equitability

(Pielou) indices were calculated using the number of strains per species and for each of the studied islands. Values are presented in tables (Table 2).

Through the Figure 3, it was possible to observe the frequency of tested colonies or strains for a specific biochemistry protocol, allowing the identification of particular species of bacteria.

The Tamboretes Island presented higher diversity (2.36) of pathogenic bacteria, followed by Moleques do Sul (2.10) and Lobos Islands (1.35). Regarding the values recorded for the Margalef richness index, Moleques do Sul had the highest value (2.87), followed by Tamboretes (2.55) and Lobos (1.14). The Pielou equitability index was higher for Tamboretes (0.92), followed by Moleques do Sul (0.79) and Lobos (0.51).

The similarity was calculated using the Jaccard and Bray-Curtis methods. The values are presented in the Tables 3a and 3b, which allow for a comparison of the microbial diversity among the three islands.

Through Cluster charts of the three islands, a comparative analysis between the similarity indices show that Jaccard index demonstrated greater proximities between Tamboretes and Moleques do Sul (Figure 4a), while for the Bray-Curtis indices, there was greater similarity between Lobos and Tamboretes (Figure 4b).

The results of this study suggest a strong association between pathogenic bacteria and the species *L. dominicanus* caught on three islands of the coast of Santa Catarina, during their reproductive period. Of the 39 samples collected on the islands, all were positive for some pathogenic species of bacteria.

Table.1 This table presents the identified Species of Bacteria (Coloring by Gram Method – or +) in the population of *Larus dominicanus* between May 2011 and November 2013. The values of (n) show the number of isolated strains tested by a specific biochemistry protocol, while the values of (%) reflect such number in terms of percentage.

Moleques do Sul Island				Lobos Island				Tamboretetes Island			
Species (sp.)	Gram	n	%	Species (sp.)	Gram	n	%	Species (sp.)	Gram	n	%
<i>Citrobacter koseri</i>	-	11	11.96	<i>Citrobacter koseri</i>	-	11	14.10	<i>Citrobacter amalonatius</i>	-	1	0.92
<i>Enterobacter aerogenes</i>	-	2	2.17	<i>Enterobacter cloacae</i>	-	1	1.28	<i>Citrobacter freundii</i>	-	6	5.50
<i>Enterobacter agglomerans</i>	-	1	1.09	<i>Proteus mirabilis</i>	-	18	23.08	<i>Citrobacter koseri</i>	-	11	10.09
<i>Escherichia coli</i>	-	2	2.17	<i>Providencia rettgeri</i>	-	1	1.28	<i>Enterococcus spp.</i>	+	4	3.67
<i>Klebsiela pneumoniae</i>	-	4	4.35	<i>Salmonella enteritidis</i>	-	19	24.36	<i>Escherichia coli</i>	-	2	1.83
<i>Leclercia adecarboxylata</i>	-	1	1.09	<i>Staphylococcus aureus</i>	+	28	35.90	<i>Klebsiela pneumoniae</i>	-	4	3.67
<i>Proteus vulgaris</i>	-	4	4.35	**	**	**	**	<i>Proteus mirabilis</i>	-	18	16.51
<i>Salmonella cholerae suis</i>	-	1	1.09	**	**	**	**	<i>Proteus vulgaris</i>	-	4	3.67
<i>Salmonella enteritidis</i>	-	19	20.65	**	**	**	**	<i>Salmonella enteritidis</i>	-	19	17.43
<i>Salmonella typhy</i>	-	4	4.35	**	**	**	**	<i>Salmonella typhy</i>	-	4	3.67
<i>Shigella spp.</i>	-	7	7.61	**	**	**	**	<i>Shigella spp.</i>	-	7	6.42
<i>Staphylococcus aureus</i>	+	28	30.43	**	**	**	**	<i>Staphylococcus aureus</i>	+	28	25.69
<i>Staphylococcus epidermidis</i>	+	7	7.61	**	**	**	**	<i>Yersinia sp.</i>	-	1	0.92
<i>Yersinia pseudotuberculosis</i>	-	1	1.09	**	**	**	**	**	**	**	**
		92	100.00			78	100.00			109	100.00

Table.2 Values for Diversity, Richness and Equitability Index Calculated for the Number of isolated Strains between May 2011 and November 2013.

	Moleques do Sul	Lobos	Tamboretetes
Diversity (H')	2.1058	1.3592	2.3618
Richness (D_{mg})	2.8750	1.1477	2.5579
Equitability (J')	0.7979	0.5150	0.9208

Table.3 Values for the Jaccard (a) and Bray-Curtis (b) Similarity Index for the Comparison between the Number of isolated Strains between May 2011 and November 2013.

a)

	Moleques do Sul	Lobos	Tamboretetes
Moleques do Sul	1.00	0.16	0.33
Lobos	0.16	1.00	0.31
Tamboretetes	0.33	0.31	1.00

b)

	Moleques do Sul	Lobos	Tamboretetes
Moleques do Sul	1.00	0.16	0.33
Lobos	0.16	1.00	0.31
Tamboretetes	0.33	0.31	1.00

Fig.1 The Map above shows the Three Islands where were Conducted the Field Activities, located at Santa Catarina coast, South Brazil.

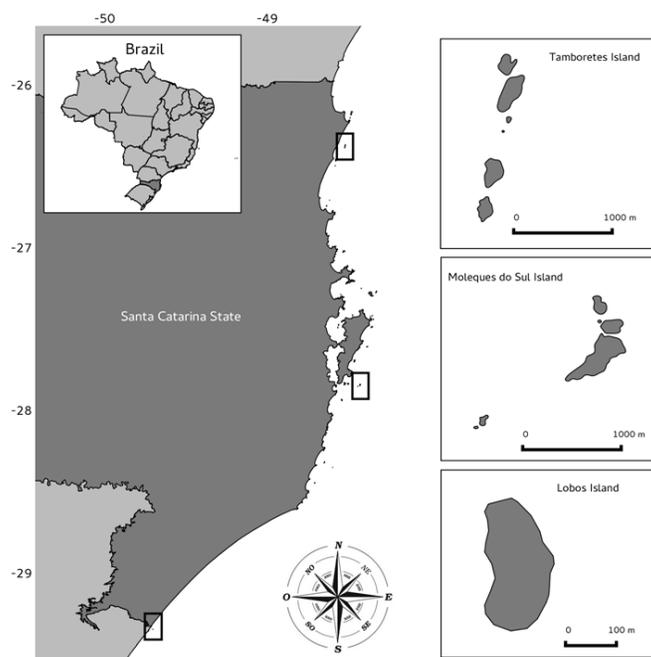


Fig.2 Percentage of Samples obtained on the Three Islands (a), and Percentage of the Number of Identified Species of Bacteria from Cloacal Swabs of *Larus dominicanus* the three Islands between May 2011 and November 2013.

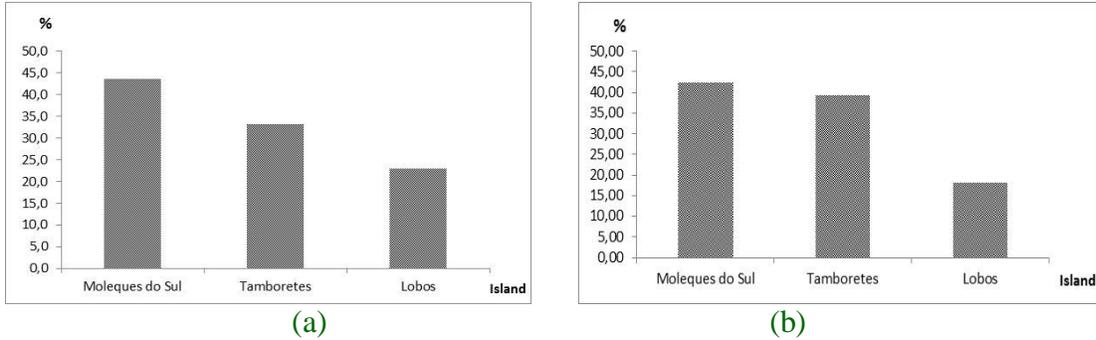


Fig.3 The figure above shows the Frequency among the isolated Strains. The records are for Moleques do Sul Island (a), Lobos Island (b) and Tamborettes Islands (c).

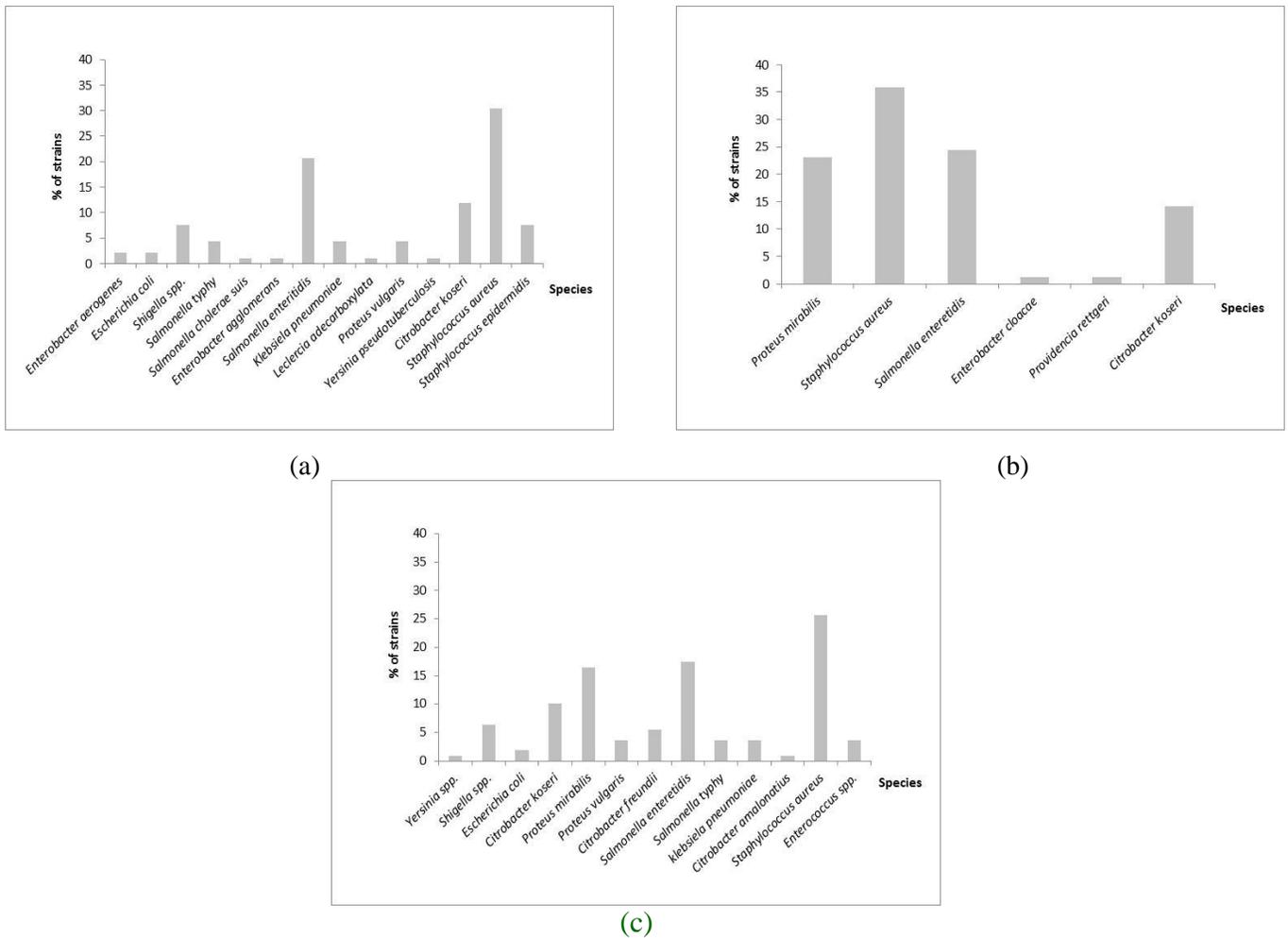
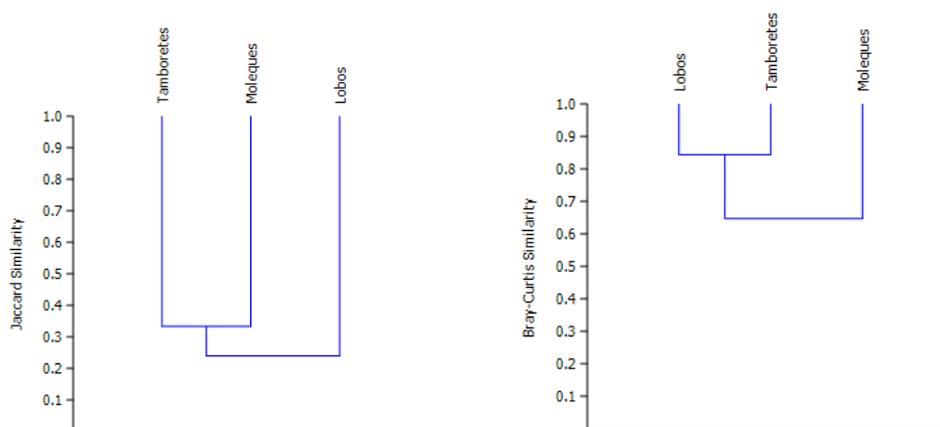


Fig.4 Similarity of Species identified from isolates among Moleques do Sul, Tamboretes and Lobos islands, through the Jaccard (a) and Bray-Curtis (b) indices.



Thirty-two bacterial species were recorded from cloacal samples of the species *Sula leucogaster* on Ilhas Moleques do Sul and Ilhas Tamboretes, prompting for care in the spread of diseases through biological vectors (Castro-Silva *et al.*, 2011). The isolation of campylobacteria from *Larus ridibundus*, including species that are zoonotic enteropathogens, indicates that wild birds potentially serve as a reservoir for human infection (Debruyne *et al.*, 2010). The isolation of campylobacteria from *L. ridibundus*, including species that are zoonotic enteropathogens, indicates that wild birds potentially serve as a reservoir for human infection (Debruyne *et al.*, 2010). In Canada, a study conducted by Kinzelman *et al.*, (2008), it was observed that in 30% of samples, pathogenic bacteria were found, indicating the possibility of the species *Larus argentatus* and *Larus delawarensis*, two very common gulls on the Canadian coast, to be vectors of diseases transmitted to humans.

The kelp gull is a very common bird on the entire Brazilian coast, and thus, it is important to acknowledge these gulls as a reservoir and potential vector of disease

transmission. How this pathogenic microorganisms affects the kelp gull is a question to observe, because is unknown the effects on reproductive success for example.

Although still very incipient, seabirds microbiota studies can serve as an important control tool of pathological and epidemiological problems (Debruyne *et al.*, 2010; Santos *et al.*, 2012). The presence of species potentially hazardous to human health, such as the species of the genus *Salmonella*, combined with the ability of the birds to *offset al.*,ong the coast and even enter the coastal territory facilitates the transport of antibiotic-resistant strains and possible human pathogens (Potti *et al.*, 2002). Colonies of the species *Salmonella enteritidis* were observed in samples from all the islands, and it is the second highest occurring bacteria, in terms of percentage, among the colonies found. In studies of Kinzelman *et al.*, (2008), few samples of *Salmonella* were found, but they do not rule out the potential risk of seabirds to act as a reservoir of these bacteria.

Siebert *et al.*, (2012) investigating seabirds and coastal birds populations in Germany

suggests that pathogenic bacteria such as *Clostridium perfringens* and *Escherichia coli* could harm the health of these birds by interfering, for example, on the reproductive success through premature mortality of juveniles and neonates. The authors Castro-Silva *et al.*, (2011) warn of the high risk of contamination of both public and animal health by bacteria of the type *Salmonella*, often associated with enteric and/or septic processes (Benskin *et al.*, 2009). *Salmonella enteritidis* and *Salmonella cholerae suis* stand out among the species with the greatest epidemiological significance.

Both species are important causes of infectious diseases that afflict individuals and their ability to mutate and antibiotic resistance. The production of cytotoxins, the presence of endotoxins and resistance to conventional antibiotics are among the main virulence factors associated with infections by different serotypes of *Salmonella* in animals (Ribeiro *et al.*, 2010). *Salmonella enteritidis* was recorded in samples from all the islands, being the second largest group in representativeness.

These results suggest evidence that the gulls could act as a reservoir for such bacterial strains, becoming potential vectors of transmission of the associated diseases. In the Argentinean Patagonia region, the population of the species grows at alarming levels, causing numerous ecological problems and associated diseases binding problems, as they can take advantage of anthropogenic food available in that region (Schiavini and Yorio, 1995; Yorio *et al.*, 1998). Thus, careful strategies should be taken in order to allow the proper management of the species *L. dominicanus*.

The identification of the bacteria associated with seabirds is the first step towards differentiation between commensal and/or

pathogenic microbiota present in the species *L. dominicanus*, which in turn can be affected by many variables such as diet, environment, and also the contact with other infected birds. Some authors point out that the habitat and feeding patterns can be decisive for the composition of the bacteria that will be associated with these birds (Mills *et al.*, 1999; Waldenstrom *et al.*, 2002; Maul *et al.*, 2005; Benskin *et al.*, 2009; Rose *et al.*, 2009; Debruyne *et al.*, 2010; Chryssanthou *et al.*, 2011; Santos *et al.*, 2012; Sarker *et al.*, 2012).

In Argentina, there is strong concern about the increase in populations of *L. dominicanus*, as it is often observed feeding on garbage dumps and landfills close to their breeding sites. In those articles, the authors emphasize the strong association between pathogenic bacteria and parasites, and externalize their concern about these animals acting as vectors of pathogen transmission to humans (Schiavini and Yorio, 1995; Yorio and Quintana, 1996; Frere *et al.*, 2000; Diaz *et al.*, 2011).

The most representative species in our study, in order of importance and similar to all the islands, were the species *Staphylococcus aureus*, *Salmonella enteritidis* and *Citrobacter koseri*. Although some studies analyzing the microbiota associated with seabirds and shorebirds demonstrated the presence of *Escherichia coli* (Schiavini and Yorio, 1995; Yorio and Quintana, 1996; Kinzelman *et al.*, 2008; Castro-Silva *et al.*, 2011), the results for this species was lower than 2%, observed in samples from Tamborettes and Moleques do Sul Islands.

This species is usually associated with environments contaminated with human waste and such correlation could indicate unsanitary environment. Tamborettes

Islands, located in the northern-central coast of Santa Catarina, is close to the largest industrial center of the state, the city of Joinville, and records of *Escherichia coli* of these samples may suggest evidence of contamination of these sites. This bacterial species is one of those species in which strains multidrug-resistant to antimicrobials have rapidly emerged, due to its wide environmental distribution and coverage in the possibilities of placement to man and animals (Rose *et al.*, 2009).

The species *L. dominicanus* is often observed feeding near the fishing companies in the state of Santa Catarina, where fish are handled, often without proper sanitization methods. Close contact of the gull at these places could potentiate the risk of cross contamination to humans through contaminated fish consumption (Ebert and Branco, 2009). Studies showed that the gulls contribute to the contamination of oysters in nature and in cultivation farms which could there by potentiate the transmission of diseases to humans (Albarnaz *et al.*, 2007).

However, attention should also be given to other species of bacteria identified, which are related to the type of feeding of the *L. dominicanus*. Lobos and Tamboretes Islands are close to major urban centers, only a few miles away from the coast, and there are reports that the gulls could take food from anthropogenic origin deposited in those cities landfills. As an opportunistic feeding (Schiavini and Yorio, 1995; Yorio *et al.*, 1998; Ebert and Branco, 2009), it often resorts to alternative sources to get their food out of the breeding season, thus contributing to the carrying of harmful species of bacteria to the Santa Catarina islands. As an example, the genus *Enterobacter*, observed in samples collected on Lobos and Moleques do Sul, is responsible for various infectious diseases, and in birds, *Staphylococcus aureus* is

known to cause various diseases such as chronic osteomyelitis and acute septicemia (Skeeles, 1997).

Microbial diversity at Tamboretes showed the highest values for Shannon ($H' = 2,3618$), which may represent evidence of environmental contamination, since this island is close to the largest industrial center in the state of Santa Catarina. This information is similar to the results observed for microbiota of shorebirds in the estuary of the Tagus, near Lisbon in Portugal (Santos *et al.*, 2012). For Jaccard, a qualitative index, greater similarity occurred between Tamboretes and Moleques do Sul Islands, whereas Bray-Curtis (quantitative index) approaches Lobos and Tamboretes islands regarding its microbial diversity.

This type of information is very important for the environmental management, whereas the diversity of the pathogenic bacteria could be correlated to aspects of environmental contamination. With the exception of Tamboretes, both Moleques do Sul and Lobos are located in areas of ecological importance. Moleques do Sul is inserted in a state park called Parque Estadual da Serra do Tabuleiro, home to endemic species (Cherem *et al.*, 2011), whereas Lobos Island is a nursery for several marine species, especially whales that migrate from the Argentinian Patagonia to give birth in warmer waters (Cesconetto *et al.*, 2011).

Other species of bacteria identified in this study may also contribute to the transmission of severe diseases. In Bangladesh, *Staphylococcus* spp., *Bacillus* spp., *Escherichia coli*, *Proteus* spp. and *Salmonella* spp. were isolated from oral swabs, cloacal swabs and faecal samples of apparently healthy six different species of water birds (Sarker *et al.*, 2012).

Studies show that samples of *Yersinia* sp., when inoculated intragastrically in man, have the ability to invade multiple organs and tissues, where they remain for varying periods (Medeiros *et al.*, 1987). The presence of bacteria such as *Yersinia pseudotuberculosis* and *Klebsiella pneumoniae* found in samples of *L. dominicanus* feces reinforce theories that these birds serve as vectors of contamination and development of resistant strains of these species. The isolated species *Providencia rettgerii* a kind of Gram-negative bacteria belonging to the Enterobacteriaceae family. They are opportunistic pathogens in humans and are mainly related to urinary tract infection, especially in patients with extensive burns or probes. This species is still related to cases of urinary tract infection and is also a serious problem of hospital contamination, as observed in the probes used in patients (Sousa *et al.*, 2004).

In general, the observation of pronounced levels of pathogenic bacteria leads to hypotheses for explaining the marine environment contamination. According to these results, in the Southern Argentinean town of Ushuaia, the Antarctic Peninsula, Antarctic Islands and the Falkland Islands were found a wide variety of yeast and bacteria associated with gulls, probably reflecting pollution of coastal seawater from urban waste and other human activities (Chryssanthou *et al.*, 2011).

Thus, the presence of contaminated *L. dominicanus* suggests that areas near the studied islands are unhealthy environment and/or such areas have their conservation status changed. Noteworthy is that these islands are located in key areas of environmental conservation, such as the Parque Estadual da Serra do Tabuleiro and a marine reserve called Reserva Marinha do Arvoredo. This study is a first for the coastal

region of Santa Catarina and contributes with updated information on the microbiota present in kelp Gulls. Research of this magnitude are very important for the management of the marine environment, allowing effective prophylactic measures to combat possible pathogenic outbreaks.

Acknowledgements

This research was founded by Conselho Nacional de Ciência e Tecnologia (CNPQ), Brazil, under a posgraduate fellowships number 141672 / 2011-0. All the logistics fields activities were conducted by support of Centro Universitário Leonardo da Vinci (UNIASSELVI), Clube de Observadores de Aves do Vale Europeu (COAVE) and Projeto Aves Marinhas, CTTMar – UNIVALI.

References

- Albarnaz, J.D., Toso, J., Correa, A.A., Simoes, C.M.O., Barardi, C.R.M. 2007. Relationship between the contamination of gulls (*Larus dominicanus*) and oysters (*Crassostrea gigas*) with Salmonella serovar Typhimurium by PCR-RFLP. *Int. J. Environ. Res.*, 17(2): 133 – 140.
- Altwegg, R., Crawford, R.J.M., Underhill, L.G., Martin, A.P., Whittington, P.A. 2007. Geographic variation in reproduction and survival of kelp gulls *Larus dominicanus* vetula in southern Africa. *J. Avian Biol.*, 38: 580-586.
- Barbieri, E. 2008. Variação sazonal do gaivotão (*Larus dominicanus*) durante o ano de 2005 no estuário de Cananéia-Iguape-Ilha Comprida, São Paulo, Brasil. *Biota Neotrop.*, 8(2): 97–102.

- Bege, L.A.R., Pauli, B.T. 1989. As aves nas ilhas Moleques do Sul – Santa Catarina: aspectos da ecologia, etologia e anilhamento de aves marinhas. FATMA Press, Florianópolis.
- Benskin, C. Mc.W.H., Wilson, K., Jones, K., Hartley, I.R. 2009. Bacterial pathogens in wild birds: a review of the frequency and effects of infection. *Biol. Rev.*, 84: 349–373.
- Blaber, S.J.M., Milton, D.A., Smith, G.C., Farmer, M.J. 1995. Trawl discards in the diets of tropical seabirds of the northern Great Barrier Reef, Australia. *Mar. Ecol. Prog. Ser.*, 127: 1-13.
- Boersma, P.D., Clark, J.A., Hillgarth, N.2001. Seabird Conservation. In. Shereiber, E.A., Burger, J. (eds). *Biology of Marine Birds*. CRC Press, New York, pp. 559-574.
- Branco, J.O., Fracasso, H.A.A., Barbieri, E. 2009. Breeding biology of the kelp gull (*Larus dominicanus*) at Santa Catarina coast, Brazil. *Ornitologia Neotropical*, 20: 409–419.
- Branco, J.O. 2004. Aves marinhas das Ilhas de Santa Catarina. In. Branco, J. (ed). *Aves marinhas e insulares brasileiras: biologia e conservação*. UNIVALI Press, Itajaí, pp. 15-36.
- Branco, J.O. 2003. Reprodução das aves marinhas nas ilhas costeiras de Santa Catarina, Brasil. *Rev. Brasil Zoo.*, 20(4): 619-623.
- Branco, J.O. 2001. Descartes da pesca do camarão sete-barbas como fonte de alimento para aves marinhas. *Ver. Brasil Zoo.*, 18(1): 293-300.
- Branco, J.O. 2000. Avifauna associada ao estuário do Saco da Fazenda. *Rev. Brasil Zoo.*, 17(2): 387-394.
- Burger, J., Gochfeld, M. 1996. Family Laridae (gulls). In. (Hoyo, J. del, Elliott, A., Sargatal J. (eds). *Handbook of the Birds of the World*. Lynx Editions, Barcelona, Spain, pp. 572–623.
- Castro-Silva, M.S., Manoel, F.C., Krueger, J., Barreiros, M.A.B., Branco, J.O. 2011. Identificação de bactérias potencialmente patogênicas a humanos presentes em *Sula leucogaster* (Suliformes: Sulidae), no litoral de Santa Catarina, Brasil. *Revista Brasileira de Ornitologia*, 19(4): 520-524.
- Cesconetto, C., Lopes, G.S., De-Rose-Silva, R., Groch, K.R., Seyboth, E. 2011. Ocorrência de baleias francas austrais (*Eubalaena australis*) na área central da área de proteção ambiental da Baleia Franca durante a Temporada Reprodutiva de 2010. 3º Congresso Brasileiro de Biologia Marinha (CBBM), Natal, Rio Grande do Norte.
- Cherem, J.J., Graipel, M.E., Tortato M., Althoff, S, Bruggemann, F., Matos, J., Voltolini, J.C., Freitas, R., Illenseer, R., Hoffman, F., Ghizoni, I.R., Bevilacqua, A., Reinicke, R., Salvador, C.S., Filippini, A., Furnari, N., Abati, K., Moraes, M., Moreira, T., Oliveira-Santos, L., Kuhnen, V., Maccarini, T., Goulart, F., Mozerle, H., Fantacini, F.2011. Mastofauna terrestre do Parque Estadual da Serra do Tabuleiro, estado de Santa Catarina, sul do Brasil. *Biotemas.*, 24(3): 73-84.
- Chryssanthou, E., Wennberg, H., Bonnedahl, J., Olsen, B. 2011. Occurrence of yeasts in faecal samples from Antarctic and South American seabirds. *Mycoses*, 54: 811–815.
- Debruyne, L., Broman, T., Bergstro, S., Olsen, B., On, S.L.W., Vandamme, P. 2010. *Campylobacter volucris* isolated from black-headed gulls

- Larus ridibundus*. *Int. J. Syst. Evol. Microbiol.*, 60: 1870–1875.
- Diaz, J.I., Cremonte, F., Navone, G.T. 2011. Helminths of the kelp gull *Larus dominicanus* from the northern Patagonian coast. *Parasitol. Res.*, 109: 1555–1562.
- Dobbin, G., Haribaron, H., Daoust, P., Haribaron, S., Heaney, S., Coles, M., Price, L., Muckle, C. 2005. Bacterial flora of free-living Double-crested cormorant (*Phalacrocorax auritus*) chicks on Prince Edward Island, Canada, with reference to enteric bacteria and antibiotic resistance. *Comp. Immunol. Microbiol. Infect. Dis.*, 28:71-82.
- Ebert, L.A., Branco, J.O. 2009. Variação sazonal na abundância de *Larus dominicanus* (Aves, Laridae) no Saco da Fazenda, Itajaí, Santa Catarina. *Iheringia*. 99: 437-441.
- Frere, E., Gandini, P., Peck, M.R. 2000. Gaviota Cocinera (*Larus dominicanus*) como vector potencial de patógenos en la costa patagónica. *Hornero*, 15: 93-97.
- Furness, R.W., Monaghan, P. 1987. Seabird Ecology. Chapman & Hall Press, New York.
- Furness, R.W. 2003. Impacts of fisheries on seabirds communities. *Sci. Mar.*, 67: 33-45.
- Borboroglu, P.G., Yorio, P. 2004. Effects of microhabitat preferences on kelp gull *Larus dominicanus* breeding performance. *J. Avian Biol.*, 35: 162-169.
- Kelley, T.R., Pancorbo, M.W., Barnharts, H.M. 1998. Antibiotic resistance of bacterial litter isolates. *Poult. Sci.*, 77: 243-247.
- Kinzelman, J., McLellan, S., Amick, A., Preedit, J., Scopel, C., Olapade, O., Gradus, S., Singh, A., Sedmak, G. 2008. Identification of human enteric pathogens in gull feces at Southwestern Lake Michigan bathing beaches. *Can. J. Microbiol.*, 54: 1006–1015.
- Louzao, M., Igual, J.M., McMinn, M., Aguilar, J.S., Triai, R., Oro, D. 2006. Small Pelagic Fish, trawling discards and breeding performance of the critically endangered balearic shearwater: improving conservation diagnosis. *Mar. Ecol. Prog. Ser.*, 318: 247-254.
- Lu, J., Idris, U., Harmon, B., Hofacre, C., Maurer, J.J., Lee, M.D. 2003. Diversity and succession of the intestinal bacterial community of the maturing broiler chicken. *Appl. Environ. Microbiol.*, 69: 6816–6824.
- Martinez-Abraín, A., Raquel, M., Oro, D. 2002. Demersal trawling as a food source for Western Mediterranean seabirds during the summer. *ICES J. Mar. Sci.*, 59: 529-537.
- Maul, J., Ganghi, J., Farris, J. 2005. Community-level physiological profiles of cloacal microbes in songbirds (Order: Passeriformes): variation due to host species, host diet, and habitat. *FEMS Microbiol. Ecol.*, 50: 19–28.
- Medeiros, B.M.M. de, Shimizu, M.T., Falcao, D.P. 1987. Período de infectividade de animais inoculados experimentalmente com *Yersinia* sp. *Rev. Saúde Pública.*, 21: 261-264.
- Mills, T., Lombardo, M., Thorpe, P. 1999. Microbial colonization of the Cloacae of nestlings tree swallows. *Auk.*, 116: 947–956.
- Palmgren, H., Sellin, M., Bergstrom, S., Olsen, B. 1997. Enteropathogenic bacteria in migrating birds arriving in Sweden. *Scand. J. Infect. Dis.*, 29: 565–568.
- Potti, J., Moreno, J., Yorio, P., Briones, V., Borboroglu, G.P., Villar, S.,

- Ballesteros, C. 2002. Bacteria divert resources from growth for magellanic penguin chicks. *Ecol. Lett.*, 5: 709–714.
- Ribeiro, M.G., Fernandes, M.C., Paes, A.C., Siqueira, A.K., Pinto, J.P.A.N., Borges, A.S. 2010. Caracterização de sorotipos em linhagens do gênero *Salmonella* isoladas de diferentes afecções em animais domésticos. *Pesq. Vet. Bras.*, 30(2): 155-160.
- Rose, J.M., Gast, R.J., Bogomolni, A., Ellis, J.C., Lentell, B.J., Touhey, K., Moore, M. 2009. Occurrence and patterns of antibiotic resistance invertebrates off the Northeastern United States coast. *FEMS Microbiol. Ecol.*, 67: 421–431.
- Santos, S., Pardal, S., Proença, D., Lopes, R., Ramos, J., Mendes, L., Morais, P. 2012. Diversity of cloacal microbial community in migratory shorebirds that use the Tagus estuary as stopover habitat and their potential to harbor and disperse pathogenic microorganisms. *FEMS Microbiol. Ecol.*, 82: 63–74.
- Sarker, M.A.H., Jahan, M., Parvin, M.N., Malek, M.A., Hossain, M.T. 2012. Identification of bacterial flora isolated from apparently healthy water birds of dhaka zoo of Bangladesh. *Bangl. J. Vet. Med.*, 10(1&2): 21-26.
- Schiavini, A., Yorio, P. 1995. Distribution and abundance of seabird colonies in the Argentine sector of the beagle channel, Tierra del Fuego. *Marine Ornithol.*, 23: 39-46.
- Schiefler, A.F., Soares, M. 1995. Estudo comparativo da avifauna das praias de Navegantes e Laguna, Santa Catarina. *Biotemas*, 7(1&2): 31-45.
- Sick, H. 1997. *Ornitologia Brasileira*. Nova Fronteira Press, Rio de Janeiro.
- Siebert, U., Schwemmer, P., Guse, N., Harder, T., Garthe, S., Prenger-Berninghoff, E., Wohlsein, P. 2012. Health status of seabirds and coastal birds found at the German North Sea coast. *Acta. Vet. Scand*, 54(43): 2-8.
- Silva, N. da, Junqueira, V.C.A., Silveira, N.F. de A. 2010. Manual de métodos de análise microbiológica de alimentos e água. Varela Press, São Paulo.
- Skeeles, J.K. 1997. Staphylococcosis. In: Calnek, B.W. (ed). *Diseases of Poultry*. Iowa State University Press, Ames, pp. 247-253.
- Skov, H., Dunrinck, J. 2001. Seabirds attraction to fishing vessels is a local process. *Mar. Ecol. Prog. Ser.*, 214: 289-298.
- Sousa, O.V., de, Vieira, R.H.F., dos S, Menezes, F.G.R. de, Reis, C.M.F. dos, Hofer, E. 2004. Detection of *Vibrio parahaemolyticus* and *Vibrio cholerae* in oyster, *Crassostrea rhizophorae*, collected from a natural nursery in the Cocó river estuary, Fortaleza, Ceará, Brazil. *Rev. Inst. Med.*, 46(2): 59-62.
- Sullivan, B.J., Timothy, A.R., Bugoni, L. 2006. Seabird mortality on factory trawlers in the Falklands islands and beyond. *Biol. Cons.*, 131: 495-504.
- Twentyman, C.M., Alley, M.R. 1999. Circovirus-Like infection in a southern black-backed gull *Larus dominicanus*. *Avian Pathol.*, 28: 513 – 517.
- Waldenstrom, J., Carlsson, T., Hasselquist, I., Achterberg, D., Wagenaar, R., Olsen, J. 2002. Prevalence of *Campylobacter jejuni*, *Campylobacter lari* and *Campylobacter coli* in different ecological guilds and taxa of migrating birds. *Appl. Environ. Microbiol.*, 68: 5911–5917.
- Yorio, P., Bertelotti, M., Gandini, P., Frere, E. 1998. Kelp Gulls *Larus dominicanus* breeding on the

- Argentina coast: population status and relationship with coastal management and conservation. *Marine Ornithol.*, 26: 11-18.
- Yorio, P., Quintana, F. 1996. Efectos del disturbio humano sobre una colonia mixta de aves marinas en Patagonia. *Hornero.*,14: 60-66.
- Zador, S.G., Punt, A.E., Parrish, J.K. 2008. Population impacts of endangered short-tailed albatross bycatch in the Alaskan trawl fishery. *Biol. Cons.*, 141: 872-882.

How to cite this article:

Luis A. Ebert, Júlio C. Schlemper, Márcia R. Pelisser, Bianca de A. Pereira, Marcus A.C. da Silva and Joaquim O. Branco. 2016. Pathogenic Bacteria Associated with Kelp Gull *Larus dominicanus* (Charadriiformes, Laridae) on the Coast of Santa Catarina State – Brazil. *Int.J.Curr.Microbiol.App.Sci.*5(5): 458-473.
doi: <http://dx.doi.org/10.20546/ijcmas.2016.505.048>