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Detection of Potential Human Pathogenic Bacteria Isolated From Feces of Two Colonial Seabirds Nesting on Isla Rasa, Gulf of California: Heermann's Gull (*Larus heermanni*) and Elegant Tern (*Thalasseus elegans*)

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Abstract

Some seabird species have shown to be carriers of human pathogenic bacteria, given their occurrence in contaminated coastal areas. This in turn may pose a health risk to humans and become a possible factor in the spread of infectious diseases. In this scenario, we studied whether potential human pathogenic bacteria of genera *Escherichia*, *Vibrio*, and *Staphylococcus* can be traced in the Heermann's Gull and the Elegant Tern, two seabirds from Isla Rasa, Gulf of California. To this end, freshly deposited fecal droppings from both seabirds were collected and evaluated through several methods (standard bacterial methods, multiplex PCR, 16S rRNA sequencing, and matrix-assisted laser desorption ionization time-of-flight mass spectrophotometry). We identified *Escherichia coli* isolates, however, no evidence of pathogenicity was found. The analysis of the 16S rRNA sequences allowed the identification of isolates related to Vibrio *alginolyticus* and *Vibrio parahaemolyticus*, both previously reported as human pathogens. Also, analysis of 16S rRNA and matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) leads to the identification of isolates related to *Staphylococcus saprophyticus*, *Staphylococcus sciuri*, and *Staphylococcus aureus*, also reported as human pathogens. However, the identified bacteria do not represent any of the species or strains that most importantly affect humans. This study represents the first approach to the identification of pathogenic microorganisms in seabirds nesting in the central region of the Gulf of California; however, it was limited to only two species of seabirds and a few microorganisms. Therefore, monitoring and surveillance work like this should be continued and expanded to other species.

Keywords

Thalasseus elegans, *Larus heermanni*, pathogenic bacteria, 16S rRNA, anthropogenic disturbance, Gulf of California

Introduction

Seabirds are a group of vertebrates of the class Aves that have been adapted to live in the marine habitat, from which they extract most of their food. They usually breed in colonies established on islands or, less frequently, along the coastal areas or even, and very seldom, inland (Elphick, 2016). Due to their colonial nesting habit, large amounts of their feces concentrate on certain land areas and water surfaces. For example, some seabird species living close to coasts have shown to be

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carriers of human pathogenic bacteria, contributing to the local microbial load (Frere, Gandini, & Martínez-Peck, 2000). An important contribution to organic fecal contamination in coastal areas is due to animals and human wastes discharges, which contain potential pathogenic bacteria, including species of *Aeromonas*, *Escherichia*, *Salmonella*, *Staphylococcus*, *Campylobacter*, and *Vibrio*, among others (Ferns & Mudge, 2000; Lévesque, Brousseau, Bernier, Dewailly, & Joly, 2000; Meerburg, Koene, & Kleijn, 2011; Moriarty et al., 2011; Wither, Rehfisch, & Austin, 2005). In addition, these microorganisms are discharged into the ocean, posing a risk to the health of people consuming seafood or to swimmers on contaminated beaches and are a determining factor in the spread of infectious diseases (Stewart et al., 2008). However, despite the numerous studies on the microbiota present in seabirds, only a few have reported the prevalence of human pathogenic bacteria. For example, Moriarty et al. (2011) evaluated the water quality of recreational beaches in New Zealand, in terms of the presence of bacteria with pathogenic potential such as enterococci, *Escherichia coli*, *Campylobacter*, *Salmonella* spp., and *Cryptosporidium* spp. To this end, the feces of Mallard Duck (*Anas platyrhynchos*), Black Swan (*Cygnus atratus*), Canada Goose (*Branta canadensis*), and gulls (scientific names were not provided) were evaluated. The results showed the occurrence of *E. coli* in 95% of all seabird samples (with Mallard ducks being the most frequent), whereas in the case of enterococci, the occurrence was 94%. In addition, the authors reported that *Campylobacter* was present in 59% of the gulls and 29% of the ducks, while *Salmonella* was not present in any of the seabird species studied. In another study, Meerburg et al. (2011) evaluated *E. coli* levels in bird feces in recreational waters in the Netherlands. Fecal samples of 85 birds were used, consisting of Coots (*Fulica atra*), Black-backed gull (*Larus fuscus*), European herring gull (*Larus argentatus*), Black-headed gull (*Larus ridibundus*), and Common goose (*Anser anser*). A higher occurrence of *E. coli* was observed in gulls than in geese and coots, a result attributed to opportunistic behavior in omnivorous gulls, while the other birds are known to be herbivores. Also in Europe, Wither et al. (2005) described the impact of a group of birds (vaguely described as gulls, feral pigeons, waders, and starlings) on the quality of recreational waters on the Fylde coast (Northwest England). This led the authors to conclude that some birds are an important source of fecal water contamination. On the other hand, a recent study along the eastern coasts of the Mediterranean island of Sicily by Monticelli, Decembrini, Bergamasco, and Caruso (2019) suggested that seabirds such as the Yellow-legged gull (*Larus michahellis*) may also contribute as a source of antibiotic resistant enterococci in coastal lagoons and adjacent marine areas.

In Mexico, events such as climate change, human activity, pollution, and the introduction of invasive species, among others, have directly affected the health of vertebrate species in marine ecosystems (International Panel on Climate Change, 2014). Thus, the study of the relationship between pathogenic microorganisms with seabirds and marine mammals can be used to evaluate a certain aspect of the conservation status of marine ecosystems, as well as the degree of contamination due to human activity. Although there is a general interest for the study of the microbiota present in the vertebrates of the Gulf of California (a refuge region for migratory seabirds, shorebirds and waterbirds, and a harbor to large marine mammals), there are no studies describing the presence of human pathogenic microorganisms in migratory seabirds. Therefore, in this study, we explored the occurrence of potential pathogenic bacteria from genera *Escherichia*, *Vibrio*, and *Staphylococcus* in two seabirds species: the Heermann's Gull (*Larus heermanni*) and the Elegant Tern (*Thalasseus elegans*) and investigated to what extent these bacteria are shared between the two species.

Methods

Study Area

Isla Rasa is a small island within the Gulf of California (28° 49′ 33.45″ N and 112° 58′ 55.64″ W), it has an area of only 0.68 km^2, and is part of the Area of Protection of Flora and Fauna Islands of the Gulf of California (Comisión Nacional de Áreas Naturales Protegidas, 2000). The island is one of the youngest in the gulf, with an age estimated at approximately 10,000 years (Carreño & Helenes, 2002), is volcanic in origin, and consists mainly of basaltic rock with poor accumulated soil (Velarde, Wilder, Felger, & Ezcurra, 2014). It is in one of the most productive marine ecosystems in the world, and the lack of land predators provides the island with a nesting site for several seabirds, including both the Heermann's Gull and the Elegant Tern, which were examined for potential pathogenic bacteria given their wide distribution range along the western Pacific coasts of north, central, and south America. The island was also subject to intense human activity: first due to guano-mining operations between around 1870 and 1910 and later by the harvesting of seabird eggs in commercial quantities (Bowen, 2000). This activity continued until federal protection as a wildlife reserve was declared (Diario Oficial de la Federación, 1964). To date, the island is still visited mainly by fishermen, tourists, and researchers. Moreover, these seabirds have had contact with humans along the North American Pacific coasts (including the Baja California peninsula coasts, the Gulf

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of California, and as far as Guatemala) for the Heermann's Gull, and along north to south Pacific coasts (from California to northern Chile) for the Elegant Tern. This has been ongoing at least for the last 150 years, exposing these seabirds to potentially become reservoirs for human pathogenic bacteria.

Sample Collection, Isolates Preservation, and Detection of Potential Human Pathogenic Bacteria

A total of 95 freshly deposited fecal droppings from both the Heermann's Gull (44) and the Elegant Tern (51) were collected on June 2012. To do this, individuals were carefully observed while resting on rocks near the shore, and fecal droppings were collected with swabs immediately after they were deposited by these birds. All samples were stored in Amies medium and taken to the laboratory within 4 days after collection. Later, each swab was resuspended in a sterile saline solution (0.9% v/v, 5 mL), and the isolated colonies of presumptive *E. coli*, *Vibrio* spp., and *Staphylococcus* spp. were tested and cultured as described in the Supplementary Material. The obtained isolates were preserved in Todd Hewitt Broth + glycerol 40% at −20°C. The molecular and MALDI-TOF approaches for the detection and identification of potential human pathogenic bacteria are also described in the Supplementary Material. The list of primers used in this study for the 16S rRNA sequencing of *Vibrio* and *Staphylococcus* spp. are described in Supplementary Table 1, and bacterial species identified in the Heermann's Gull (*L. heermanni*) and the Elegant Tern (*T. elegans*) are described in Table 1.

Results

E. coli *Diarrheagenic Groups*

We identified 12 *E. coli* isolates from Heermann's Gulls and three from Elegant Terns. These 15 isolates indicated that at least 27.3% and 5.9% of the sampled Heermann's Gull and the Elegant Tern, respectively, hosted *E. coli* (Table 1). However, even though this bacterium was found in many individuals of the two species of seabirds, no evidence of pathogenicity was found, as no PCR products of diarrheagenic *E. coli* pathotypes was observed: From all the genes evaluated to detect molecular pathotypes (*it*, *st*, *bfpA*, *eaeA*, *stx1*, *stx2*, and *ial*), none of them were detected in any of these isolates. Therefore, no *E. coli* pathogenic isolates were detected in either seabird species.

Vibrio *spp. Identification*

The 16S rRNA BLAST comparison allowed the identification of three *Vibrio* spp. isolates, from which two were isolated from Heermann's Gulls and one from an Elegant Tern (Table 1). Specifically, isolates related with Vibrio *alginolyticus* were found in two individuals, both Heermann's Gulls, and an isolate related with *Vibrio parahaemolyticus* was found in one Elegant Tern individual (Table 1). Both *Vibrio* species have been previously reported as human pathogens (e.g., Daniels et al., 2000; Dziuban et al., 2006). The same BLAST comparisons also showed that none of the isolates sequenced and identified as *Vibrio* in this study yield BLAST hits with *Vibrio cholerae* (accession no. LC011481), a species of special concern given its pathogenicity among humans and fishes.

Staphylococcus *spp. MALDI-TOF and Molecular Identification*

The 16S rRNA sequences allowed the identification of 13 *Staphylococcus* spp. isolates, from which six were isolated from Heermann's Gulls and seven different isolates were isolated from Elegant Terns (Table 1). BLAST comparisons revealed that two 16S rRNA sequences (isolates TEA029a and TEA043) are closely related to *Staphylococcus saprophyticus* (100% identity and 100% Query Cover), five (isolates LHC001, LHA014, LHA027, TEC013a, and TEC013b) to *Staphylococcus sciuri* (99.77% identity and 100% Query Cover), and six (isolates LHA010, LHA035a, LHA035b, TEA035b, TEA053a, and TEA053b) to *Staphylococcus aureus* (100% identity and 100% Query Cover in five cases and 99.77% identity and 99% Query Cover in one case) (Table 1). Therefore, all 13 16S rRNA sequences were grouped together with the three *Staphylococcus* species (*S. aureus*, *S. Sciuri* and *S. saprophyticus*) that have been reported as human pathogens (e.g., Kitai et al., 2005; Nemeghaire et al., 2014; von Eiff, Peters, & Heilmann, 2002). On the other hand, although six out of 13 *Staphylococcus* isolates were positive to coagulase, none of them revealed the occurrence of the methicillin-resistant gene (*mecA*), as no PCR product of 310 bp was observed. Finally, the *Staphylococcus* species identified by mass spectrometry (using the MALDI-TOF technology) were *S. saprophyticus*, *S, sciuri,* and *S. aureus*, the same that in the BLAST comparisons (Table 1).

Discussion

Globally, bacteria that are regularly transmitted by the consumption of contaminated water are responsible for many infectious and parasitic gastrointestinal diseases, causing approximately 2.2 million deaths per year (Ramirez-Castillo et al., 2015). Examples of these are *Vibrio* spp., which causes the most common infection known as gastroenteritis (including the etiological agent of cholera); some *E. coli* strains, a common

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Table 1. Bacterial Species Identified in the Heermann's Gull (*Larus heermanni*) and the Elegant Tern (*Thalasseus elegans*).

Species	Heermann's Gull ($n = 44$)	Isolate (GenBank accession number)	Highest BLAST hit (% of identity, % Query Cover)	Elegant Tern ($n = 51$)	Isolate (GenBank accession number)	Highest BLAST hit (% of identity, % Query Cover)
Escherichia coli[a]	12 (27.3%)	LHC001 LHC002 LHC003 LHA005 LHA014 LHA019 LHA026 LHA029 LHA031 LHC034 LHA038 LHC040		3 (5.9%)	TEA001 TEA041 TEA048	
Vibrio *alginolyticus*	2 (4.5%)	LHA009 (MF142797) LHA024 (MF142799)	*V. alginolyticus* strain CMFRI/VAI-39 (98.53%, 100%) *V. alginolyticus* strain PC14 (96.91%, 100%)			
Vibrio parahaemolyticus				1 (2%)	TEA047 (MF142801)	*V. parahaemolyticus* strain SC2 (94.77%, 100%)
Staphylococcus sciuri[b]	3(6.8%)	LHC001 (MF142784) LHA014 (MF142786) LHA027 (MF142787)	*S. sciuri* strain PTXIII (99.77%, 100%) *S. sciuri* strain PTXIII (99.77%, 100%) *S. sciuri* strain PTXIII (99.77%, 100%)	2 (3.9%)	TEC013a (MF142790) TEC013b (MF142791)	*S. sciuri* strain APBSMLB223 (99.77%, 100%) *S. sciuri* strain APBSMLB223 (99.77%, 100%)
Staphylococcus aureus[b]	3 (6.8%)	LHA010 (MF142785) LHA035a (MF142788) LHA035b (MF142789)	*S. aureus* KG-22 DNA (100%, 100%) *S. aureus* KG-22 DNA (100%, 100%) *S. aureus* KG-22 DNA (100%, 100%)	3 (5.9%)	TEA035b (MF142793) TEA053a (MF142795) TEA053b (MF142796)	*S. aureus* KG-22 DNA (100%, 100%) *S. aureus* KG-22 DNA (100%, 100%) *S. aureus* clone WZ061 (99.77%, 99%)
Staphylococcus saprophyticus[b]				2 (3.9%)	TEA029a (MF142792) TEA043 (MF142794)	*S. saprophyticus* strain FDAARGOS_355 (100%, 100%) *S. saprophyticus* subsp. saprophyticus strain YNC-12 (100%, 100%)

The number after each bacteria species name is the number of seabird individuals that hosted the microorganism's isolate. Percentages in parenthesis refer to the proportion of seabird individuals hosting bacteria with respect to the whole seabird species sample. Names of individual isolates and GenBank accession numbers (of corresponding 16S rRNA sequences) are provided. n = number of sampled individuals from each seabird.

[a]Although *E. coli* isolates were identified in both Heermann's Gull and the Elegant Tern, none of them was found to be pathogenic (see *E. coli* diarrheagenic groups in the "Results" section).

[b]The same *Staphylococcus* species were identified by mass spectrometry (with the MALDI-TOF technology).

cause of gastrointestinal diseases (Fricker, 2003); and some *Staphylococcus* spp. strains, which are opportunistic pathogens (Koksal, Yasar, & Samasti, 2009). These bacteria have been found hosted by vertebrates, which provide the necessary nutrients and adequate conditions for their development and survival (Stewart et al., 2008). Birds are among the vertebrates identified as reservoirs of pathogenic bacteria that directly affect the human health. Also, reports from several countries (Frere et al., 2000; Meerburg et al., 2011; Moriarty et al., 2011; Wither et al., 2005) indicate that wild birds are a major contributor of microbial loads in recreational waters (beaches, riverbanks, lakes, etc.), which could pose a health risk to humans recreating in such areas. In this study, we found that despite the current human activity in the Midriff Island Region in the Gulf of California, the evidence of human pathogenic bacteria is of little importance. In fact, the pathogenic bacteria we found do not represent any of the species or strains within *E. coli*, *Vibrio*, and *Staphylococcus* that most importantly affect humans. Rather, they belong to species or strains that represent little concern, in terms of the severity of the diseases caused by them, or the number of affected people.

Some gull species (such as *Larus delawarensis*, *Larus dominicanus*, *L. fuscus*, *L. argentatus*, *Larus glaucescens,* and *L. ridibundus*) are considered to have a great potential in affecting water quality and transmitting Enterobacteriaceae pathogens such as some *E. coli* strains (Alderisio & DeLuca, 1999; Fogarty, Haack, Wolcott, & Whitman, 2003; Frere et al., 2000; Meerburg et al., 2011; Trapp, 1979). A possible explanation for this is that some species of gulls have been shown to use urban landfills, dumpsters, and fishing boat discards to feed themselves. This in turn may have increased the potential for them to ingest different pathogens. In this sense, as no evidence was gathered in this study on the occurrence of pathogenic *E. coli* strains in the Heermann's Gull, it may be assumed that this relationship between anthropogenic waste and this seabird has not sufficiently documented yet. Given that this gull has no commensal habits with humans of the magnitude that other species of gulls have done, such as those already mentioned (among others), the probability that it acquires bacterial pathogenic strains is much lower. However, further surveillance may shed light on whether this is the result of current conditions or a consequence of limitations in our study. In contrast, Elegant Terns do not show an opportunistic feeding behavior, and there are no reports of them feeding at dumpsters, which limits the potential for contact between these seabirds and pathogenic bacteria. In this study, from the four *E. coli* pathotypes investigated (which are among the most important diarrheagenic *E. coli* groups found in Mexico) (Lopez-Saucedo et al., 2003), none of them were detected in Elegant Terns.

On the other hand, gulls have also been reported as carriers of cholera-causing pathogenic bacteria (*V. cholerae*). For example, Ogg, Ryder, and Smith (1989) demonstrated that out of 1,131 samples of 20 aquatic bird species sampled in Colorado and Utah, among which were the California Gull (*Larus californicus*), the Franklin's Gull (*Larus pipixcan*), and the Ring-billed Gull (*L. delawarensis*), 17% were carriers of this bacterium. This high prevalence may have occurred because these birds feed in water bodies contaminated with human feces carrying *V. cholerae*. In our study, we found three isolates grouped together with *Vibrio* species *V. parahaemolyticus*, and *V. alginolyticus*, which are of human health concern. However, we did not identify *V. cholerae* pathogenic isolates in any individual of the two species of seabirds. Prevalence of isolates was very low, as the one related to *V. alginolyticus* was identified in only two Herrmann's Gull individuals, while the one related to *V. parahaemolyticus* was identified in only one Elegant Tern. On the other hand, *V. alginolyticus* is considered a pathogenic species of both humans and marine invertebrates (Gomez-Leon, Villamil, Lemos, Novoa, & Figueras, 2005). In humans, its pathogenicity is low; however, it can cause various diseases such as intestinal and skin infections, along with ulcers and abscesses. It can also cause ear and eye infections such as conjunctivitis (Dziuban et al., 2006). Finally, *V. parahaemolyticus* is responsible for infections associated with consumption of raw or undercooked shellfish, as well as with contaminated food. It is recognized as a common cause of foodborne illness (e.g., gastroenteritis) in Asia and the United States of America, and many people exposed to it have been diagnosed with gastroenteritis and other disorders, mainly associated with exposed wounds (Daniels et al., 2000; Dziuban et al., 2006).

Three species of *Staphylococcus* were also identified: *S. saprophyticus, S. sciuri,* and *S. aureus*. Their identification was consistent among all three methods used: conventional microbiological identification, mass spectrometry (MALDI-TOF), and 16S rDNA sequencing. These species have been reported as part of the normal microbiota of air, soil, and water (Faria, Vaz-Moreira, Serapicos, Nunes, & Manaia, 2009); however, they have also been isolated from fecal droppings in the Ring-billed Gull (*L. delawarensis*) (Lévesque et al., 2000), and from skin and mucous membranes of many animals, including humans and birds (Becker, Skov, & von Eiff, 2015). All three species have been reported as human pathogens, and their frequency was comparable to that of all other pathogenic bacteria reported in this study (Table 1). Both *S. sciuri* an *S. aureus* occurred at similar proportion in either seabird species; however, *S. saprophyticus* was isolated from only two Elegant

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Terns and none from Heermann's Gull. *S. sciuri* was in three Heermann's Gulls and in two Elegant Terns, while *S. aureus* was also in three individuals from both Heermann's Gull and Elegant Tern. *S. sciuri* is a urinary pathogen that causes both upper and lower urinary tract disease, among other diseases in the immunocompetent patients (Nemeghaire et al., 2014). Several authors have reported that *S. sciuri* is frequently associated with domestic and wild animals; however, its clinical importance in recent years has increased, as it can cause disease in humans and other hosts (Dakić et al., 2005; Stepanovic et al., 2001; von Eiff et al., 2002). On the other hand, *S. aureus* is considered a pathogenic bacterium for humans when virulence factors that alter host health are present. This mainly occurs through food poisoning, which is a consequence of enterotoxins produced by some *S. aureus* strains (Kitai et al., 2005). Another problem associated with the presence of *Staphylococcus* strains is that many have already acquired resistance to antibiotics such as methicillin, which makes their medical treatment difficult. In our study, none of the identified coagulase-positive *Staphylococcus* isolates revealed the presence of the *mecA* gene. Consequently, it is unlikely that the seabirds from which samples were taken have been in contact with antibiotics such as methicillin and β-lactams.

This study represents the first approach to the search and identification of human pathogenic bacteria in seabirds nesting in the central region of the Gulf of California. However, it was limited to only two species of seabirds and a few microorganisms. In addition, it is not clear at what point the identified microorganisms become harbored by these seabirds (i.e., if pathogens are part of the microbiota acquired during the chick stages at the birth sites or are acquired by them along their migrations). Moreover, new economic activities, as well as the expansion of human settlements and population growth, exert an increased pressure in the zone, which forces the seabirds to modify their behavior patterns and increases their contact with humans. Therefore, monitoring and surveillance work like this should be continued and expanded to other species to cope with the need for baseline studies, surveys, and inventories and to better understand the ecological consequences of human population growth.

Implication for Conservation

The results of this study demonstrate that, even though *E. coli* was identified in association with the Elegant Terns and the Heermann's Gull, no human pathogenic isolates (considering the most common pathotypes already described in Mexico) were found. On the other hand, we found isolates related to *V. alginolyticus*, *V. parahaemolyticus*, *S. saprophyticus*, *S. sciuri*, and *S. aureus*, all of them previously reported as human pathogens; however, we did not find evidence of species of special concern given its pathogenicity among humans, such as *V. cholerae*. Only *S. aureus* have been reported among those that represent a major risk for human health; however, as no occurrence of the *mecA* gene was detected, we inferred that the resistance to methicillin and all β-lactams was absent from our isolates. Altogether, the results of this study indicate that the occurrence of pathogenic bacteria is a current phenomenon at least in the sampled area, it is probably linked to the increase in human activity over the last two centuries and may also probably associated with an increase in water contamination. Therefore, monitoring and surveillance work like this should be continued and expanded, both in terms of the geographic area sampled and to other relevant species (not only seabirds but also marine mammals such as the California sea lion [*Zalophus californianus*] could be more suited). That will allow to cope with the need for baseline studies, surveys, and inventories and to better understand the ecological consequences of human population growth.

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Declaration of Conflicting Interests

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