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Gulls identified as major source of fecal pollution in coastal waters: A microbial source tracking study

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HIGHLIGHTS

- Gulls could be an important source of pollution in coastal environments.
- Microbiological quality of bathing water was assessed in the Berlenga Island beach.
- BOX-PCR fingerprinting profiles of *E. coli* were used to identify sources of beach contamination.
- Gull feces constitute a major source of fecal contamination of the bathing water of Berlenga Island.

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ABSTRACT

Gulls were reported as sources of fecal pollution in coastal environments and potential vectors of human infections. Microbial source tracking (MST) methods were rarely tested to identify this pollution origin. This study was conducted to ascertain the source of water fecal contamination in the Berlenga Island, Portugal. A total of 169 *Escherichia coli* isolates from human sewage, 423 isolates from gull feces and 334 water isolates were analyzed by BOX-PCR. An average correct classification of 79.3% was achieved. When an 85% similarity cutoff was applied 24% of water isolates were present in gull feces against 2.7% detected in sewage. Jackknifing resulted in 29.3% of water isolates classified as gull, and 10.8% classified as human. Results indicate that gulls constitute a major source of water contamination in the Berlenga Island. This study validated a methodology to differentiate human and gull fecal pollution sources in a real case of a contaminated beach.

1. Introduction

Fecal contamination of coastal areas is a worldwide concern, as waterborne disease outbreaks are increasing in the last years and water quality is degraded (Santo Domingo and Edge, 2010; WHO, 2009). Gulls have been reported as one of the major sources of fecal pollution in coastal environments (Lee et al., 2013; Converse et al., 2012; Lu et al., 2008). The health risks associated with gull feces are largely unknown. However, due to migratory character and feeding habits of the birds, gulls are recognized as important vehicles for spread of bacterial pathogens, impacting coastal areas. Previous studies have shown that gull feces carry potentially pathogenic bacteria like *Campylobacter* spp. (Kinzelman et al., 2008), *Salmonella* spp. (Kinzelman et al., 2008; Literák et al., 1992), *Listeria* spp. (Fenlon, 1985) and verocytotoxin-producing *Escherichia coli* O157 (Wallace et al., 1997). Additionally, gulls have been considered an important vector of dissemination of antimicrobial resistance genes because of their association with human activities (Čížek et al., 2007; Dolejská et al., 2007). Recently, an investigation demonstrated that decreasing gull populations lead to a significant reduction in fecal indicator bacteria (FIB) densities and enteric human pathogens in coastal water (Converse et al., 2012).

FIB monitoring is typically used to evaluate water quality and is considered as a measure of risk. However, FIB detection and counting only indicate whether or not the aquatic system is impacted by fecal contamination and to some extent allow to predict the occurrence of pathogens, as they are found in the feces of most warm-blooded animals (Wu et al., 2011; Payment and Locas, 2011; Scott et al., 2002). Thus, FIB monitoring does not provide any information about the source of such pollution (McLellan, 2004). This is particularly dramatic in coastal

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areas, where recreational activities can put humans into contact with enteric microorganisms from different sources.

To determine the source of contamination (human, livestock, wildlife or other) is an important issue since the illness risk from exposure to contaminated water may be significantly different depending on the origin of contamination (Parveen et al., 2001). In fact, it is believed that fecal contamination from wildlife presents lower human health risks when compared to human feces. Even so, wildlife species can also carry human pathogens such as *Campylobacter* spp., *Toxoplasma gondii*, and *Giardia* spp. that may pose a health risk to humans as well (Santo Domingo and Edge, 2010). The identification of the pollution source, whether it's of animal or human origin, is essential so that local authorities can implement the appropriate management measures to restore water quality and limit the risk of disease as the revised Bathing Europe Water Directive (2006/7/EC) requires. For the same purpose, it is important to establish robust and reproducible methods allowing the discrimination between fecal contaminations of human origin from those of wildlife origin.

During the past years several microbial and chemical markers have been suggested as methods for this purpose (Simpson et al., 2002). These techniques, referred as a whole as microbial source tracking (MST) methods, emerged to provide not only a more accurate assessment of water quality but also to determine the source of contamination. The use of these methods is based on the assumption that the characteristics of intestinal microbiota depend on the specific host. MST methods can be divided in culture-dependent and culture-independent methods. Culture-dependent methods are less expensive and include an enrichment step, increasing the number of analyzed organisms. Most of these methods are based on FIB for which there are available data on prevalence and correlation with the occurrence of pathogens. Culture-dependent methods can be based on genotypic or phenotypic characteristics (USEPA, 2005). The phenotypic methods include antibiotic resistance analysis (ARA), carbon-source utilization profiling (CUP) and, fatty acid methyl ester (FAME) profiling (Field and Samadpour, 2007; Santo Domingo and Edge, 2010). The genotypic approaches include repetitive elements PCR (rep-PCR), amplified fragment length polymorphism (AFLP), pulse-field gel electrophoresis (PFGE) and ribotyping (USEPA, 2005; Field and Samadpour, 2007). The choice of the methodology depends on the characteristics of the specific aquatic system as well as on the putative sources of contamination. PCR-based methods for DNA fingerprinting, namely rep-PCR, of *E. coli* isolates have been reported as accurate means to identify sources of fecal contamination on water systems and are one of the most common methods used for this purpose (Carlos et al., 2012; Stoeckel et al., 2004; Guan et al., 2002; Parveen et al., 2001; Dombek et al., 2000). Rep-PCR fingerprints can be generated using repetitive extragenic palindromic (REP) sequences (35–40 bp), enterobacterial repetitive intergenic consensus (ERIC) sequences (124–127 bp) or the BOX elements (154 bp) (Versalovic et al., 1994).

E. coli has been extensively used as a target in MST studies since, like other FIB, it is a natural member of the intestinal microbiota of warm-blooded animals and its intra-specific diversity is host-dependent (Ma et al., 2011; Parveen et al., 2001).

The Berlenga Island belongs to an archipelago located at 5.7 miles from the Portuguese coastline, classified as Natural Reserve since 1981 and more recently (2011) as Biosphere Reserve by UNESCO. Its insular nature, geographical location and climate, complemented by a limited human presence, contributed to the preservation and speciation of some terrestrial and marine flora and fauna (Queiroga et al., 2008; Amado et al., 2007).

Despite the management measures applied, a clear expansion of the population of the most abundant seabird species in the island known as yellow-legged gull (*Larus cachinnans*), has been observed over time (Queiroga et al., 2008). In the summer season, a high number of tourists visit the island for diverse recreational activities and seafood harvesting.

In the last few years, fecal contamination of water has been detected in the beach located at the Berlenga Island. We hypothesized that the origin of the fecal contamination was either the only human-derived sewage effluent in the island or the feces of the gulls' population; this takes in consideration the scarcity of other animal species. Given the characteristics of such a specific environment, we decided to set as main objective of our study to test a source tracking methodology to identify the source of fecal pollution detected in the water of the Berlenga Island beach. We intend to validate this methodology to discriminate between gull and human microbial contaminations in this confined environment, which can be further applied in different geographic regions namely by environmental authorities. A robust analysis of the fecal pollution origins in coastal waters with timely results would provide relevant knowledge to implement an efficient management plan.

2. Material and methods

2.1. Sampling site

The Berlengas archipelago is located in the Portuguese continental shelf 5.7 miles northwest of Cape Carvoeiro (Peniche, Portugal) and consists of a main island, the Berlenga Island, Estelas and Farilhões islands and some small islets and rocks (Fig. 1). Given its location and the influence of Nazaré Canyon, Berlenga Island is an important habitat for several marine and terrestrial species and a nesting area of sea birds which constitute the dominant local fauna (Queiroga et al., 2008). The gulls are the species most represented with a large population of yellow-legged gull (*L. cachinnans*). The most likely sources of fecal contamination in the island are gulls and a human-derived effluent from a service area that includes a restaurant and public sanitary facilities. This wastewater is collected on a settlement tank and is not subjected to any further treatment. From this point, the wastewater is discharged near the coastline of the island.

2.2. Sample collection

Sampling was performed every two weeks between May and September 2011, the period during which water quality is monitored by official authorities. Access to the island in the rest of the year is difficult depending on the meteorological conditions. In each sampling date were collected: i) 5 composite fecal samples, each composed of 5 to 10 individual fresh gull droppings scattered on the beach; ii) 250 mL of raw human sewage taken from the effluent of the sanitary infrastructures of the island; and iii) 2 L of sea water collected at high-tide and about 30 cm from the surface, in a location where the water column was at least 1 m deep. Every week *E. coli* and *Enterococcus* counting was performed on 100 mL of water, according to Portuguese law (DL 135/2009) to assess the quality of coastal bathing waters. All samples were collected with sterile containers, kept on ice and processed within 8 h after collection.

2.3. Isolation and confirmation of E. coli

After homogenization of the fecal composite samples, 1 g of the mixture was used to prepare a suspension in 0.9% NaCl. From this suspension 10-fold serial dilutions were prepared. Human sewage samples were also diluted in 10-fold increments. One milliliter of fecal and sewage dilutions and 10, 30 and 50 mL of seawater samples were filtered through 0.45 μm membrane filters (Millipore, Bedford, USA). The filters were placed on Chromocult Coliform Agar (CCA) (Merck, Darmstadt, Germany) plates and incubated at 37 °C during 18 to 24 h. Characteristic colonies colored dark-blue to violet were selected from one plate and streaked onto the surface of CCA plates and incubated at 37 °C for 18 to 24 h. Streaking on CCA and incubation was repeated as