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Potential for gulls to transport bacteria from human waste sites to beaches

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HIGHLIGHTS

- Gull cloacae and feces samples contained the human specific marker, HF 183.
- Markers for gull and human contamination showed spatial and temporal overlap.
- Radio-telemetry supports potential for gulls to disperse human-associated microbes.
- Gulls may act as transport vectors of human pathogens.
- Gull4 was a more sensitive source-tracking marker when compared to Gull2.

GRAPHICAL ABSTRACT

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ABSTRACT

Contamination of recreational beaches due to fecal waste from gulls complicates beach monitoring and may pose a risk to public health. Gulls that feed at human waste sites may ingest human fecal microorganisms associated with that waste. If these gulls also visit beaches, they may serve as vectors, transporting fecal microorganisms to the beach where they may subsequently contaminate sand and water. In this study, samples collected from landfills, treated wastewater storage lagoons, and public beaches demonstrated a spatial and temporal overlap of markers for gull and human-associated microorganisms. In addition, markers for gull, fecal indicator bacteria, and the human-associated marker, HF183, were detected in gull feces and cloacae samples. Further, HF183 was detected in cloacae samples from gulls that were documented by radio-telemetry traveling between human waste sites and public beaches. This study highlights the potential for gulls that visit human waste sites to disperse human-associated microorganisms in the beach landscape.

1. Introduction

Contamination of recreational beaches with fecal waste poses a risk to human health due to the potential occurrence of human pathogens. Despite increased recognition of this risk, outbreaks of illness associated with exposure to contaminated recreational water continue (Hlavsa et al., 2015). Fecal waste coming from human sources is thought to present the greatest risk to human health, while waste from wildlife, including shore birds, is thought to be of lower risk (Schoen and Ashbolt, 2010; Soller et al., 2010). Therefore, the identification of sources of fecal contamination to recreational beaches is often included as a component of beach monitoring and mitigation efforts (Byappanahalli et al., 2015;

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Edge and Hill, 2007; Goodwin et al., 2016; Goodwin et al. 2017; Noble et al., 2006).

Several studies have revealed gulls as a significant source of contamination at beaches (Araújo et al., 2014; Converse et al., 2012; Edge and Hill, 2007; Haack et al. 2003; Lu et al., 2011a; Staley and Edge, 2016). Indeed, gull waste contains high levels of the traditional fecal indicator bacteria (FIB) used in beach monitoring (Alderisio and DeLuca, 1999; Fogarty et al., 2003; Meerburg et al., 2011), and several studies have correlated gull numbers with FIB densities in water (Converse et al., 2012; Kirschner et al., 2004; Lu et al., 2011a) and sand (Edge and Hill, 2007; Edge et al., 2010; Whitman and Nevers, 2003). More importantly, gulls are relevant to public health because they may shed bacterial pathogens (Ebert et al., 2016; Kinzelman et al., 2008; Lévesque et al., 2000; Lu et al., 2011b; Quessy and Messier, 1992; Whelan et al., 1988), antibiotic resistant bacteria (Bonnedahl et al., 2009; Dolejská et al., 2009), and viruses such as avian influenza virus (Alexander, 2000). Some of these microorganisms (e.g., campylobacters and avian influenza virus) may be endemic in gulls (Kapperud and Rosef, 1983; Webster et al., 1992), but gulls may acquire others from their environment.

Gulls are opportunistic feeders and are often attracted to easily accessible food sources linked to human activity (Ferns and Mudge, 2000). For example, in the Great Lakes region, Ring-billed gulls were found to travel up to 25 km to landfills for foraging, and anthropogenic components made up a substantial portion of their diet (Belant et al., 1998). Investigations have pointed to the potential for gulls to acquire human-associated microorganisms while foraging at sites of human refuse or waste. The prevalence of specific serotypes of *Salmonella*, including rare types, was associated with feeding at sewage treatment works (Butterfield et al., 1983; Fenlon, 1981; Fricker, 1984). *Campylobacter* occurrence, including *C. jejuni*, was directly related to refuse consumption by juvenile gulls (Ramos et al., 2010). Other studies have highlighted the potential for gulls to transport these pathogens to sites where they may then be transferred to other species. Gulls have been associated with outbreaks of salmonellosis in cattle and sheep (Butterfield et al., 1983; Coulson et al., 1983; Johnston et al., 1979) and in humans (Aavitsland and Hofshagen, 1999). Recent studies implicated gulls as vectors in the movement of *Salmonella enterica* and antibiotic-resistant *E. coli* between environmental and clinical reservoirs (Hernandez et al., 2013; Retamal et al., 2015; Toro et al., 2016; Varela et al., 2015).

The fact that wild birds, including migratory birds, have been implicated in the transmission of human pathogens (Tsiodras et al., 2008) suggests that gulls are potential reservoirs of bacterial groups used as the targets of fecal source tracking assays. However, evidence for the latter is very scarce, perhaps because most studies have been conducted in areas where larger reservoirs of human fecal waste (e.g., landfills, wastewater treatment plants) are not easily accessible to gulls. In this study, we sought to investigate whether gulls could acquire human-associated microorganisms from human waste sites and whether gulls could serve as transport vectors to recreational beaches. We examined gulls that visited, as determined by radio-telemetry, two different recreational areas that were located nearby a wastewater lagoon and two landfills, and tested them for the presence and abundance of FIB (i.e., enterococci and *E. coli*), gull- and human-associated fecal markers, and potential pathogens using qPCR assays.

2. Materials and methods

2.1. Sample sites

All sites sampled in this study were on the east coast of Lake Michigan in Ottawa and Muskegon counties, Michigan, USA (Fig. 1) and were sampled between May and August 2013. Nearshore lake water samples were collected at two public recreation beaches in Ottawa County, MI: North Beach (NB, $n = 10$) and Grand Haven City Beach (GHCB, $n = 10$). Samples were also collected at three municipal waste sites. The Muskegon County Wastewater Management System uses a land treatment process of aeration, settling, and storage on 4452 ha for treatment of domestic and industrial waste. Within this system are two, 344 ha, 5.1 billion gallon, first-stage treated wastewater storage lagoons (MWM) and the 45 ha Muskegon County Solid Waste Management System landfill (ML), which accepts septic tank, grease trap, and agricultural processing waste. The Ottawa County Farms Landfill, Coopersville, MI (CL) is a 112 ha compost facility accepting mixed domestic residential waste. All facilities are uncovered and accessible to gulls. Run-off water (CL, $n = 2$; ML, $n = 9$) was collected at both landfills. Run-off water gathered in a shallow pool at the base of the solid waste mound at ML and in a field adjacent to the solid waste mound at CL. When standing water was not present, run-off water-wetted soil slurry was collected ($n = 10$). Water samples were also collected from the wastewater storage lagoons at MWM ($n = 24$).

From May to July 2013, gulls were abundant at all sites and were nesting along a cement dyke that separates the two storage lagoons at MWM. Gulls began nesting in this region in 1974 (Ponshair, 1974) and gull nests have been counted annually on the dyke at the MWM system since 1997 (Ponshair, 2006). In June of 2013, 5516 Ring-billed gull and 11 Herring gull nests were counted (Ponshair, personal communication). Recently deposited, still moist gull feces were collected along the dyke at MWM ($n = 14$) and at NB ($n = 5$). Gull feces were collected using sterile polyester tip swabs and stored in 2 ml centrifuge tubes on ice. Additionally, gulls were captured on both beaches as part of a radio-telemetry study of gull habitat use (Jordan, 2014) and cloacal samples were collected from individual gulls ($n = 27$). To sample the cloaca (internal cavity in the digestive system), the vent was spread and a sterile polyester tip swab saturated in 1× phosphate buffered saline (PBS) solution (pH 7.5) was gently inserted up into the cloaca. The cloacal swab was then withdrawn and immediately re-submerged and stored in 1 ml PBS in 2 ml cryovials on dry ice. Feces were also collected from some of the captured gulls creating 11 paired samples of cloacal/fecal material acquired from 11 individual gulls (two captured on NB and nine captured on GHCB). Water samples were collected into sterile Whirl-Pak bags attached to a 2 m collection pole. Landfill soil slurry samples were collected into sterile 50 ml centrifuge tubes attached to a 2 m collection pole, or in some cases, soil was collected using sterile spatulas and stored in 50 ml centrifuge tubes. Field blanks during each sample collection trip included 100 ml distilled water and PBS saturated swabs.

All samples were immediately placed on ice after collection and during transportation to the laboratory. Within 6 h of collection, all water samples (30–100 ml for landfill run-off or wastewater; 600–700 ml for beach water) were processed by membrane filtration (Alm et al., 2003) and membrane filters, soil, gull feces, and gull cloacae samples were stored at −80 °C until used in DNA extractions.

2.2. DNA extraction

DNA from filtered water or from landfill soil (0.25 g wet weight) was extracted using the PowerSoil™ DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA) according to the manufacturer's instructions. For cloacae samples, each were centrifuged at 16,000 ×*g* for 9 min to concentrate the suspension, then 200–400 μl of the cloacae material were used for total DNA extraction using the PowerSoil™ DNA Isolation Kit. DNA from gull feces (0.25 g wet weight) was extracted using the UltraClean™ Fecal DNA Kit (MO BIO Laboratories) according to the manufacturer's instructions. Field blanks were also extracted.

2.3. Quantitative PCR (qPCR) assays

TaqMan-based quantitative PCR assays (Table 1) were used to test water samples (landfill run-off water, first-stage treated lagoon wastewater, and beach water), landfill run-off soil slurry, gull feces, and gull cloacae samples. TaqMan assays were performed in 25 μl containing 1× TaqMan universal PCR master mix with AmpErase uracil-*N*-glycosylase (Applied Biosystems, Foster City, CA), 0.2 μg/μl bovine