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

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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.

Graphical Abstract

Keywords

Microbial source tracking; Fecal pollution; Gull transport; Beaches; Fecal indicator bacteria

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, 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 serum albumin, 0.2 μM (final concentration) primers, 6-carboxyfluorescein (FAM)-labeled hydrolysis probe, and 2 μl of DNA template. The amplification protocol conditions included an initial incubation at 50 °C for 2 min, followed by 10 min of incubation at 95 °C, and then 40 cycles of 95 °C for 15 s and at optimum annealing temperature for each assay for 1 min. All qPCR assays were performed using a 7900 HT fast real-time sequence detector (Applied Biosystems, Foster City, CA). All assays were performed in duplicate in MicroAmp Optical 96-well reaction plates with MicroAmp Optical Caps (Applied Biosystems, Foster City, CA, USA). PCR data were analyzed using ABI's Sequence Detector software (version 2.2.2). Four independent standard curves for each qPCR assay were generated by plotting threshold cycle (C_T) values against the number of target copies corresponding to serially diluted plasmid standards purchased from IDT integrated DNA technologies (Coralville, Iowa, USA). The target copy numbers (T) were estimated by the following equation: $T = [D / (PL \times 660)] \times 6.022 \times 10^{23}$, where D (g/μl) is plasmid DNA concentration, and PL (bp) is plasmid length in base pairs. Each standard curve was generated from at least five 10-fold plasmid dilutions in triplicates. Percent amplification efficiencies were calculated by the instrument manufacturer's instructions (Applied Biosystems). Two no-template controls per PCR plate were used to check for cross-contamination. Relative copy numbers (signal intensity values) were recorded for all TaqMan assays. DNA copy numbers were standardized per respective unit for sample types (per 100 ml for water samples; per 1 g (wet weight) for landfill run-off soil slurry and gull feces; per 1 ml for cloacae suspensions).

A gull-associated SYBR green-based qPCR assay (Gull2; Lu et al., 2008), widely used in MST studies, was also used to test water sources, landfill run-off soil slurry, gull feces, and gull cloacae samples (Table 1). Reaction mixtures (25 μl total volume) for the SYBR green assay contained 1 × Power SYBR green master mix (Applied Biosystems, Foster City, CA), 0.2 μg/μl bovine serum albumin, and 0.2 μM (final concentration) of each primer, and 2 μl of DNA template. Ten-fold dilutions of each DNA extract (2 μl, final volume) were used as templates to test for PCR inhibition. The amplification protocol conditions included an initial incubation at 50 °C for 2 min, followed by 95 °C for 10 min and 40 cycles each at 95 °C for 15 s, and 64 °C annealing temperature for 1 min, followed by a melting curve analysis (i.e., from 60 to 90 °C in increments of 0.1 °C). Equipment and data analysis were performed as described above. Presence/absence readings were assigned to data generated from the Gull2 SYBR green assay based on signal intensity values.

The range of quantification for all assays, including TaqMan and SYBR green, was 10^1 to 10^5 DNA copies per reaction. Untreated domestic influent to the MWM served as a positive control for detection of HF183. For both TaqMan and SYBR green assays, all of the field blanks and no-template controls were negative, indicating no evidence of cross-contamination. A McNemar's test (Zar, 2009) on the paired gull samples was used to compare the proportion of positive HF183 samples from cloacae vs. feces. A Fisher exact test (Zar, 2009) was used to compare the proportion of positive HF183 samples among beaches, human waste sites, and gulls.

3. Results and discussion

3.1. The human-marker, HF183, was detected in gull waste samples

An objective of this study was to investigate the potential for gulls that visit human waste sites to acquire human fecal bacteria. To assess this objective the human-associated marker HF183 was used as a proxy for human fecal bacteria. Gulls at public beaches and at human waste sites were sampled to test for the presence of markers for gull, FIB, and HF183. Feces can be contaminated after excretion from the bird (IDL, 2016), and other studies have shown higher concentrations of microorganisms in cloacal material as compared to feces (Sarker et al., 2012, Tracey, 2010), therefore, we considered cloacal material to be more representative of the presence of markers in gull digestive contents and collected cloacae samples when possible. All of the markers including HF183 were detected in gull feces and cloacae samples (Table 2). From 11 individual gulls captured for radio-telemetry, both cloacal material and feces were collected (n = 11 pairs). Cloacae from paired collections returned samples that were positive for FIB (10/11) and HF183 (4/11) markers (Fig. 2). Interestingly, while feces from paired collections also had positive samples for FIB markers (7/11), none were positive for HF183. In paired collections, fewer fecal samples were positive for markers, including the gull markers, than were cloacae samples (P = 0.016, McNemar's test). These results suggest that including cloacae samples may provide information that could be missed by evaluating feces alone (Van Hoorebeke et al., 2009, Krauss et al., 2013).

The proportion of positive HF183 samples among sites differed (P = 0.020), with higher prevalence at beaches and human waste sites than within gulls. Further, the proportion of positive HF183 samples at beaches and human waste sites was equal. Concentrations of HF183 in beach water, landfills, and treated wastewater were low in comparison to concentrations in untreated influent (Table 3).

The finding of HF183 in gull cloacal material is especially interesting. The cloaca (internal cavity) is the terminus of the gull digestive system and a repository for digested material prior to expulsion as feces. Cloacal material is less likely to be contaminated by contact with the environment of the gull. Detection of HF183 in the cloaca suggests that it was ingested. To our knowledge, this is the first reported detection of HF183 in gull cloacae material. It is unlikely that HF183 can colonize gulls, but rather is present only transiently following ingestion at a contaminated site. It is possible that the detection of HF183 in gull cloacae is related to marker specificity, as cross-reactivity has been reported with nonhuman targets such as dog, chicken, and duck (Staley et al., 2012, Boehm et al., 2013). However, the HF183 TaqMan assay used in this study is considered highly specific for human source. For

example, Ahmed et al. (2016) reported a 94.6% specificity for HF183 when tested against 2966 individual nonhuman samples.

Detection of HF183 in gull cloacae and feces suggests that under some circumstances gulls may acquire human fecal bacteria. Surveys in Europe and North America have pointed to the ability of gulls to acquire potentially pathogenic bacteria including *Campylobacter* and *Salmonella* (Kinzelman et al., 2008, Lévesque et al., 2000, Quessy and Messier, 1992, Tizard, 2004). Gulls have also been shown to carry strains of *E. coli* resistant to antibiotics important in human medicine (Bonnedahl et al., 2009). Further, correlations between FIB and gull markers have implicated gulls as potential sources of FIB and pathogens to beaches (Goodwin et al., 2016). While gulls may not be common vectors of human fecal bacteria, in scenarios where there are large concentrations of gulls, or where interactions between gulls and people are likely, the public health relevance increases, particularly if the prevalence of human fecal bacteria at the sites gulls visit is high (Quessy and Messier, 1992).

3.2. Radio-telemetry corroborates potential for gulls to disperse human-associated microorganisms

To further assess the possibility of gulls acquiring and transporting human-associated bacteria, gulls (n = 27 gulls) were captured on public beaches and fit with radio-telemetry transmitters to track their movements. Following release, Jordan (2014) located gulls by radio-telemetry inland at the waste sites (24 km from capture site to CL and 38 km to MWM), as far north as Muskegon State Park (22 km from capture), and as far south as Benton Harbor, MI (105 km from capture). Five gulls were documented traveling between human waste sites (landfills or wastewater lagoons) and beaches. All samples collected from these five gulls tested positive for enterococci, *E. coli*, and Gull4 markers (Table 4). Cloacae samples from two of the five (40%) radio-tagged gulls that traveled between waste sites and beaches also tested positive for HF183, whereas only two of 22 (11%) radio-tagged gulls that did not visit waste sites during the study tested positive for HF183 (Table 4). The two cloacae samples from the tagged gulls that were positive for HF183 were negative for *Campylobacter* species, while for the other tagged gulls (negative for HF183), *Campylobacter* signals were detected.

3.3. The relationship between markers for gull and human contamination suggests a potential for gulls to act as a transport vector of human pathogens

This study hypothesizes that gulls that frequent sites with human waste can acquire human-associated bacteria and thus represent a potential vector for subsequent transport. To test this, samples were collected from sites of human waste and from public beaches and evaluated for an overlap in distribution of human-fecal bacteria and gull markers. Forty-five samples were taken of landfill run-off and wastewater storage lagoon water. *E. coli* and *Bacteroidetes* markers were frequently detected in these samples (i.e., 98%). The human marker, HF183, was found in 40% of the samples (Table 2). The campylobacter marker was also frequently detected in these samples (i.e., 96%), whereas two species of enterococci, *E. faecalis* and *E. faecium* were found in 29% and 16% of samples, respectively (Table 2). All recreational beach water samples (n = 20) tested positive for *E. coli* and *Bacteroidetes* markers while 55% of the samples tested positive for HF183 (Table 2). No recreational

beach water samples (n = 20) tested positive for the two enterococci species, while 75% of the samples tested positive for campylobacter marker and tested positive for each *E. coli* and general bacteroidetes (Table 2). These sites were also tested for the presence of gull markers. Gull2 and Gull4 were present in 42% and 60% of the samples collected at human waste sites, respectively. Most beach water samples were positive for gull markers (85% for Gull2 and 100% for Gull4). These abundances and concentrations of gull marker (Table 3) are consistent with other studies that examine waters thought to be contaminated with gull fecal waste (Cloutier and McLellan, 2017, Russell et al., 2013, Ryu et al., 2012). Our study is in agreement with the high prevalence of *E. faecalis* and *E. faecium* noted by Ryu et al. (2013) for 24 different animals including gulls, suggesting that these species are cosmopolitan (i.e., present in various hosts). *Campylobacter* species were also detected in the majority of the gull samples as well samples from the human waste sites. When coupled with the radio-telemetry findings, the qPCR data suggest that gulls traveling between human waste sites and public beaches may be dispersing human-associated microbes. Surprisingly, the prevalence of the enterococci marker and both *Enterococcus* species markers was relatively low (i.e., 30% and 0%, respectively) in the beach water samples. In contrast, 55% and 100% of the beach samples tested positive for HF183 and Gull4 markers respectively, suggesting that human and gull feces appear to be two dominant fecal contamination sources in the study area.

The presence, abundance, and distribution of gull and human markers suggest a possible role for gulls as transport vectors of human fecal bacteria. In the study area, which included both human waste sites and recreational beaches, markers associated with human fecal contamination and with gulls overlapped geographically and temporally. Other studies have also reported the presence of gull and human markers in the same environmental water samples (Byappanahalli et al., 2015, Green et al., 2012, Lauer, 2015, Russell et al., 2013). When looking at the presence of Gull4 and HF183 in waste site samples, both markers were found together in 11 samples, Gull4 alone was found in 16 samples, HF183 alone was found in 7 samples, and 11 samples did not have either marker. For public beach samples, HF183 was never found in a water sample that did not also have Gull4. Gulls are opportunistic scavengers, and it is speculated that gulls that feed at sites of human waste may acquire human-associated microbes and subsequently transport them to beach sites (Converse et al., 2012). These results are consistent with other studies that show relatedness between bacteria present in gull feces and at waste sites (Nelson et al., 2008) and with studies that indicate gulls foraging at waste sites carry potential human pathogens (Fricker, 1984, Quessy and Messier, 1992, Ramos et al., 2010). Our data do not suggest gulls are the only source of FIB or human marker in the sites sampled. However, combined with the results showing HF183 in gull cloacae, they further support that gulls that frequent human waste sites may acquire human-associated bacteria.

3.4. The Gull4 marker was found to be a more sensitive marker when compared to Gull2

The presence of gulls was evaluated in this study with two different assays (Gull2 and Gull4). The marker Gull4 was found in 100% of the cloacae samples and 87% of the feces, but Gull2 was found in only 74% of cloacae and 67% of feces (Table 2). When looking at the relationship between both markers, Gull2 was not found without Gull4. Additionally, all

of the cloacae samples from paired collections tested positive for the Gull4 marker whereas only eight (73%) of feces were positive for Gull4.

Ryu et al. (2012) also observed a high incidence of the Gull2 and Gull4 markers when tested against the feces of 255 individual gulls. Both assays also generated false positive signals when tested against non-target feces, but these were mostly associated to other avian species, suggesting that *C. marimammalium* prefers the avian gut environment. The genome of *C. marimammalium* has revealed a reduced metabolic network and several functions that are linked to a symbiotic lifestyle (Weigand et al., 2013). It should also be noted that *Catellicoccus* spp. have also been seen in migratory birds from stopover beaches simultaneously colonized by gulls (Grond et al., 2014), suggesting that fecal bacteria can be transferred between different co-inhabiting avian species.

3.5. Implications for beach management

Gulls that visit landfills, wastewater management facilities, and beaches for foraging and loafing are a possible reservoir and transport vector of FIB and human-associated bacteria. This study integrated tools of wildlife ecology and molecular microbiology to evaluate this potential. The detection of low levels of the human-associated marker, HF183, in the feces and cloacae of gulls in this study implies that gulls are able to acquire bacteria associated with human fecal waste. Further, detecting HF183 in samples collected from radio-tagged gulls with documented travel between human waste sites and public beaches suggest the potential for gulls to disperse human-associated microorganisms in the beach landscape.

Gull acquisition and transport of human bacteria to beaches may be an infrequent occurrence, however, the potential should be considered when gull abundance is high and when human waste sites are nearby and are accessible to gulls. To reduce this potential, mechanisms should be employed at waste sites to exclude gulls and should be considered at public beaches to reduce gull visitation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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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.

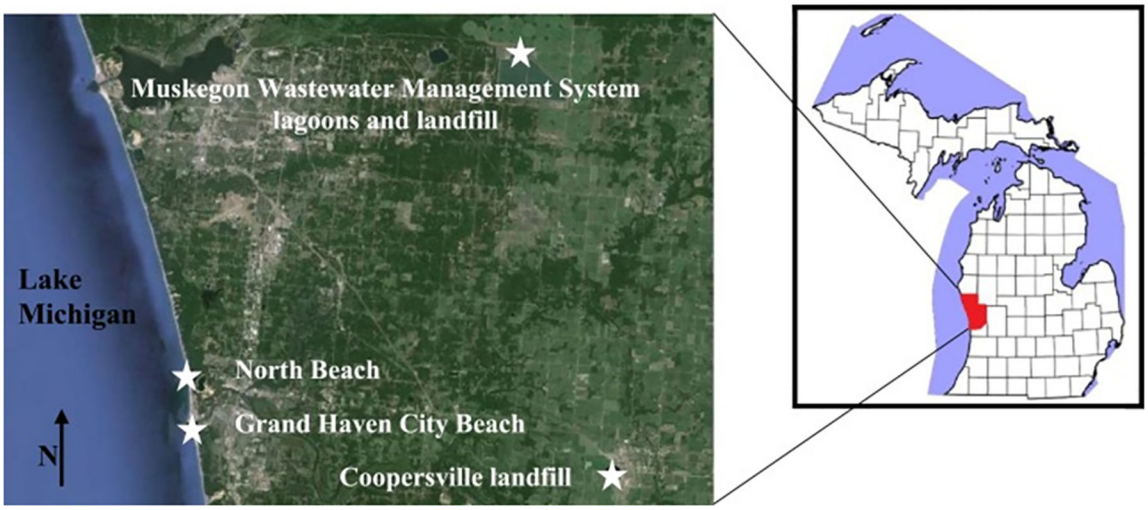

Fig. 1.

Map of study sites in western Michigan, USA. Red shading marks the outline of Muskegon and Ottawa counties of western Michigan (right map). White stars mark the site locations (left map). The Muskegon Wastewater Management System is located in Muskegon County, MI. The Muskegon Solid Waste landfill is on the property of the Muskegon Wastewater Management System. North Beach, Grand Haven City Beach, and Coopersville landfill are located in Ottawa County, MI. This map was generated using Google Earth software.

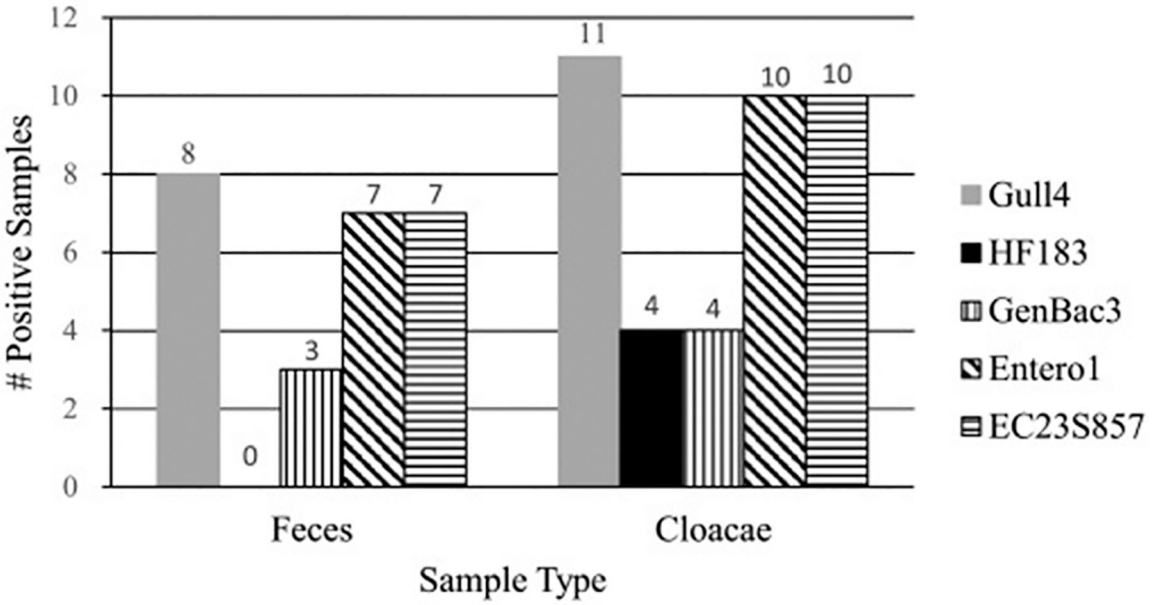

Fig. 2.

Paired feces and cloacae samples (n = 11 pairs) that had positive assays with gull-associated (Gull4), human-associated (HF183), general *Bacteroidetes* (GenBac3), enterococci (Entero1), and *E. coli* (EC23S857) markers.

Table 1.

Summary of oligonucleotide primers and probes for all qPCR assays (TaqMan and SYBR green).

Assay	Primer/probe sequence (5′ to 3′)	T (°C)[a]	Size (bp)	Reference
General *Bacteroidetes*(GenBac3) TaqMan	GenBactF3: GGGGTTCTGAGAGGAAGGT GenBactR4: CCGTCATCCTTCACGCTACT GenBactP2: 6FAM-CAATATTCCTCACTGCTGCCTCCCGTA-TAMRA	60	129	Siefring et al. (2008)
Human-associated *Bacteroidetes* (HF183) TaqMan	HF183–1F: ATCATGAGTTCACATGTCCG BthetR1: CGTAGGAGTTTGGACCGTGT BthetP1: 6FAM-CTGAGAGGAAGGTCCCCCACATTGGA-TAMRA	60	167	Haugland et al. (2010)
Gull2 SYBR green	F: TGCATCGACCTAAAGTTTTGAG R: GTCAAAGAGCGAGCAGTTACTA	64	412	Lu et al. (2008)
Gull4 TaqMan	qGull7F: CTTGCATCGACCTAAAGTTTGAG qGull8R: GGTTCTCTGTATTATGCGGTATTAGCA qGull7P[b]: FAM-ACACGTGGGTAACCTGCCCATCAGA-TAMRA	60	116	Ryu et al. (2012)
***Escherichia coli*(EC23S857) TaqMan**	F: GGTAGAGCACTGTTTtGGCA[c] R: TGTCTCCCGTGATAACtTTCTC[c] P: 6FAM-TCATCCCGACTTACCAACCCG-TAMRA	60	88	Chern et al. (2011)
General *Enterococcus*(Entero1) TaqMan	ECST748F: AGAAATTCCAAACGAACTTG ENC854R: CAGTGCTCTACCTCCATCATT GPL813TQ: 6FAM-TGGTTCTCTCCGAAATAGCTTTAGGGCTA-TAMRA	60	92	Ludwig and Schleifer (2000)
***Campylobacter* spp. (Camp2) Taqman**	campF2: CACGTGCTACAATGGCATAT campR2: GGCTTCATGCTCTCGAGTT campP2: FAM-CAGAGAACAATCCGAACTGGGACA-BHQ1–3	58	108	Lund et al. (2004)
***Enterococcus faecalis*(Faecalis1) Taqman**	FaecalF: CGCTTCTTTCCTCCCGAGT FaecalR: GCCATGCGGCATAAACTG FaecalP: 6FAM-GAGGAGTGGCGGACG-TAMRA	60	143	Santo Domingo et al. (2003)
***Enterococcus faecium*(Faecium1) Taqman**	CiumF: TTCTTTTTCCACCGGAGCTT CiumR: AACCATGCGGTTTYGATTG CiumP: 6FAM-AGTAACACGTGGGTAACCTGCCCATCAGA-TAMRA	60	141	Ryu et al. (2013)

F, forward; R, reverse; P, probe.

[a]Optimum PCR annealing temperatures were determined using temperature gradients.

[b]FAM, 6-carboxyfluorescein, fluorescence reporter dye; TAMRA, 6-carboxytetramethylrhodamine, fluorescence quencher dye.

[c]Lower case denotes deliberately mismatched base.

Table 2.

Prevalence of FIB, human- and gull-associated and pathogen markers in water, soil, gull feces, and gull cloacae samples.

Sample	*n* =	EC23S857	Entero1	GenBac3	HF183	Gull4	Gull2	Camp2	Faecalis1	Faecium1
Human waste										
CL-water	2	100%	100%	100%	50%	50%	50%	100%	100%	50%
ML-water	9	100%	78%	100%	56%	67%	44%	100%	67%	56%
ML-soil	10	90%	70%	90%	30%	20%	0%	90%	10%	10%
MWM-lagoon	24	100%	50%	100%	38%	75%	58%	96%	17%	0%
Total	45	98%	62%	98%	40%	60%	42%	96%	29%	16%
Beach										
NB-water	10	100%	40%	100%	60%	100%	80%	70%	0%	0%
GHCB-water	10	100%	20%	100%	50%	100%	90%	80%	0%	0%
Total	20	100%	30%	100%	55%	100%	85%	75%	0%	0%
Gull feces										
MWM-dyke	14	100%	100%	57%	36%	93%	57%	71%	57%	64%
NB	7	57%	43%	14%	43%	86%	86%	43%	29%	0%
GHCB	9	67%	67%	22%	0%	78%	67%	67%	44%	11%
Total	30	80%	77%	37%	27%	87%	67%	63%	53%	67%
Gull cloacae										
NB	4	50%	50%	25%	50%	100%	75%	50%	75%	0%
GHCB	23	100%	96%	30%	13%	100%	74%	57%	65%	0%
Total	27	93%	89%	30%	19%	100%	74%	56%	67%	0%

EC23S857, *E. coli*; Entero1, enterococci; GenBac3, general *Bacteroidetes*; Camp2, campylobacter; Faecalis1, *Enterococcus faecalis*; Faecium1, *Enterococcus faecium*; CL, Coopersville Landfill; ML, Muskegon County Landfill; MWM, Muskegon County Waste Management; NB, North Beach; GHCB, Grand Haven City Beach.

Table 3.

Quantification of mean positive signals for FIB, human-associated marker, and gull-associated markers in water, soil, gull feces, and gull cloacae samples.

Mean positive signal concentration (DNA/copies/unit)[a] (± standard error)					
Site-sample	**EC23S857**	**Entero1**	**GenBac3**	**HF183**	**Gull4**
Waste sites					
CL-water	$2.1 \times 10^4(\pm 1.0 \times 10^4)$	$2.4 \times 10^4(\pm 2.0 \times 10^4)$	$6.2 \times 10^3(\pm 2.0 \times 10^3)$	3.8×10^1 (NA)	5.3×10^3(NA)
ML-water	$4.6 \times 10^3(\pm 9.0 \times 10^2)$	$5.2 \times 10^5(\pm 2.0 \times 10^5)$	$2.0 \times 10^5(\pm 6.0 \times 10^4)$	$1.4 \times 10^2(\pm 1.0 \times 10^1)$	$2.0 \times 10^3(\pm 1.0 \times 10^3)$
ML-soil	$1.3 \times 10^4(\pm 6.0 \times 10^3)$	$1.9 \times 10^6(\pm 2.0 \times 10^6)$	$7.7 \times 10^4(\pm 6.0 \times 10^4)$	$1.3 \times 10^2(\pm 2.0 \times 10^1)$	$7.3 \times 10^2(\pm 2.0 \times 10^2)$
MWM-lagoon	$2.7 \times 10^4(\pm 1.0 \times 10^4)$	$2.5 \times 10^4(\pm 8.0 \times 10^3)$	$2.7 \times 10^4(\pm 5.0 \times 10^3)$	$1.7 \times 10^2(\pm 6.0 \times 10^1)$	$1.3 \times 10^4(\pm 5.0 \times 10^3)$
MWM-influent	–	–	–	3.0×10^6(single sample)	< LOQ
Beach water					
NB	$3.9 \times 10^2(\pm 1.0 \times 10^2)$	$1.1 \times 10^3(\pm 8.0 \times 10^2)$	$2.4 \times 10^3(\pm 1.0 \times 10^3)$	$5.1 \times 10^1(\pm 2.0 \times 10^1)$	$3.6 \times 10^3(\pm 3.0 \times 10^3)$
GHCB	$3.1 \times 10^2(\pm 1.0 \times 10^2)$	$8.2 \times 10^2(\pm 2.0 \times 10^2)$	$2.8 \times 10^3(\pm 2.0 \times 10^3)$	$4.0 \times 10^1(\pm 3.0 \times 10^1)$	$7.9 \times 10^2(\pm 2.0 \times 10^2)$
Gull feces					
MWM-lagoon	$6.5 \times 10^6(\pm 3.0 \times 10^6)$	$3.5 \times 10^7(\pm 2.0 \times 10^7)$	$7.8 \times 10^4(\pm 5.0 \times 10^4)$	$1.3 \times 10^2(\pm 2.0 \times 10^1)$	$8.7 \times 10^4(\pm 3.0 \times 10^4)$
NB	$5.3 \times 10^3(\pm 2.0 \times 10^3)$	$4.2 \times 10^5(\pm 4.0 \times 10^5)$	1.7×10^3(NA)	$1.2 \times 10^2(\pm 3.0 \times 10^1)$	$2.9 \times 10^6(\pm 1.0 \times 10^6)$
GHCB	$5.5 \times 10^5(\pm 4.0 \times 10^5)$	$3.2 \times 10^5(\pm 2.0 \times 10^5)$	$5.1 \times 10^3(\pm 4.0 \times 10^3)$	< LOQ	$2.4 \times 10^7(\pm 1.0 \times 10^7)$
Gull cloacae					
NB	$3.9 \times 10^3(\pm 2.0 \times 10^3)$	$5.7 \times 10^4(\pm 1.0 \times 10^4)$	1.3×10^3(NA)	$4.3 \times 10^1(\pm 5.0 \times 10^1)$	$2.8 \times 10^6(\pm 2.0 \times 10^6)$
GHCB	$8.5 \times 10^3(\pm 6.0 \times 10^3)$	$1.0 \times 10^5(\pm 1.0 \times 10^5)$	$2.1 \times 10^3(\pm 2.0 \times 10^3)$	$5.5 \times 10^1(\pm 3.0 \times 10^1)$	$5.3 \times 10^5(\pm 2.0 \times 10^5)$

EC23S857, *E. coli*; Entero1, enterococci; GenBac3, general *Bacteroidetes*. NA, only one sample of replicate collections yielded a positive signal; < LOQ, below limit of quantification. LOQs and range of amplification efficiency for each qPCR are noted in Table S1. Total number of samples (*n* =) are listed in Table 2.

[a]Sample units; water – 100 ml; soil and feces – 1 g (wet weight); cloacae – 1 ml.

Table 4.

Quantification of positive signals for FIB, human- and gull-associated and pathogen markers detected in single feces and cloacae samples from five individual gulls tracked with radio telemetry traveling between waste sites and beaches.

Mean positive signal concentration (DNA copies/unit)								
	EC23S857	**Entero1**	**GenBac3**	**HF183**	**Gull4**	**Camp2**	**Faecalis1**	**Faecium1**
Gull feces (1 g)								
[a]**GHCB to CL & GHCB to MWM**[b]	1.8×10^3	6.5×10^4	6.5×10^2	< LOQ	8.9×10^7	3.8×10^6	< LOQ	< LOQ
Gull cloacae (1 ml)								
GHCB to CL & GHCB to MWM[b]	2.0×10^3	1.1×10^4	1.8×10^2	6.3×10^1	7.0×10^3	< LOQ	< LOQ	< LOQ
GHCB to CL	8.3×10^2	4.5×10^3	< LOQ	< LOQ	2.3×10^5	1.2×10^6	< LOQ	< LOQ
GHCB to MWM & GHCB to ML	9.7×10^2	4.2×10^4	< LOQ	< LOQ	3.4×10^6	3.7×10^4	< LOQ	< LOQ
GHCB to CL	1.3×10^3	2.9×10^4	< LOQ	5.8×10^1	9.0×10^5	< LOQ	6.7×10^4	< LOQ
GHCB to CL	1.3×10^3	7.4×10^5	< LOQ	< LOQ	4.0×10^4	8.8×10^4	3.2×10^6	< LOQ

< LOQ, below limit of quantification. LOQs and range of amplification efficiency for each qPCR are noted in Table S1.

[a]Flight path, gulls were tracked flying from site A to B using radio telemetry technology where GHCB to CL is Grand Haven City Beach to Coopersville Landfill; GHCB to MWM is Grand Haven City Beach to Muskegon Wastewater Management lagoon; GHCB to ML is Grand Haven City Beach to Muskegon Landfill.

[b]Feces and cloacae samples were collected from the same gull.